

*Proceedings of the 9th*

**NATIONAL CONFERENCE ON  
WHEAT UTILIZATION RESEARCH**

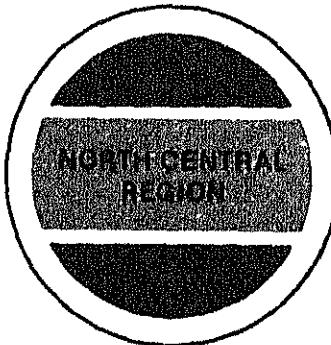
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THE NINTH NATIONAL CONFERENCE ON WHEAT UTILIZATION RESEARCH was held October 8-10, 1975, in Seattle, Washington. The objective of the biennial conference is to provide a forum for exchanging information and ideas as well as for discussing problems related to production, handling, processing, and merchandising of wheat.

Sponsors of the Conference were Agricultural Research Service, U.S. Department of Agriculture; Great Plains Wheat, Inc., and affiliated State agencies; Millers' National Federation; National Association of Wheat Growers; and Western Wheat Associates, USA, Inc., and affiliated State agencies. Chairman of the Program Committee was W. C. Schaefer, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois. Chairman of Local Arrangements was Donald F. Sundberg, Fisher Mills Inc., Seattle, Washington.

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PROTEIN CONCENTRATES  
FROM DISTILLER'S BY-PRODUCTS

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INTRODUCTION

In an effort to supply the world's ever expanding need for protein and in order to make more efficient use of the nutrient components of our food, research has gone on for a number of years in the extraction of protein from vegetable products. The number of articles on protein extraction appearing in the literature is increasing rapidly, indicating that protein has been extracted from such materials as alfalfa, soy beans, wheat and other cereal grains. Research is underway in a number of laboratories to determine the properties of these protein concentrates and to find their most efficient application in the preparation of foods for human consumption.

In the United States in 1973, approximately two billion pounds of grain were fermented in the production of ethyl alcohol (1). This grain yielded over 91 million gallons of ethanol (expressed as 200 proof) and about 430,000 tons of a by-product called distiller's dried grains and solubles (2). The distiller's dried grain is a high protein (25 to 30%) material that is used for cattle feed because its flavors and other properties make it unsuitable for human consumption. These by-product grains contained at least 200 million pounds of protein. Using alkaline extraction techniques over 100 million pounds per year of protein could be extracted from the distiller's grains with a purity of 85% and in the form of a bland powder varying in color from white to tan.

Under a grant from the National Science Foundation, studies have been made by a group of scientists and engineers at the University of Nebraska on the process conditions and process requirements for the commercial extraction of protein from distiller's by-product grains. Economic evaluations and the return on investment for alternate commercial processes have been made as a part of this grant study (3). Some pertinent results follow.

PROCESS DESCRIPTION

Figure 1 is a process flow diagram for a protein extraction plant integrated into a conventional grain alcohol production facility. Grain is fed to the plant where it is ground and cooked. Amylase is added to convert the starch and fermentation proceeds in the conventional fashion using yeast. Beer from the fermentation is fed to the beer still where ethanol is distilled off at a concentration of about 50% (100 proof). This ethanol is

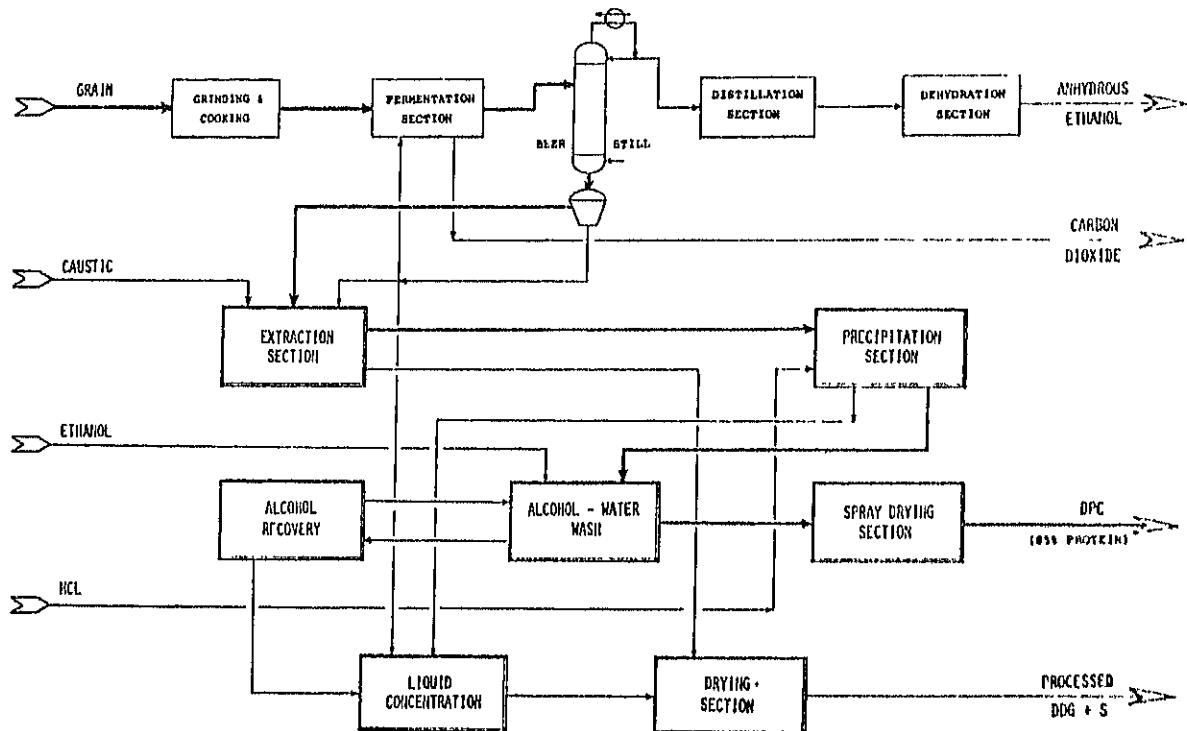


FIGURE 1 - Protein Recovery Process (Caustic Case).

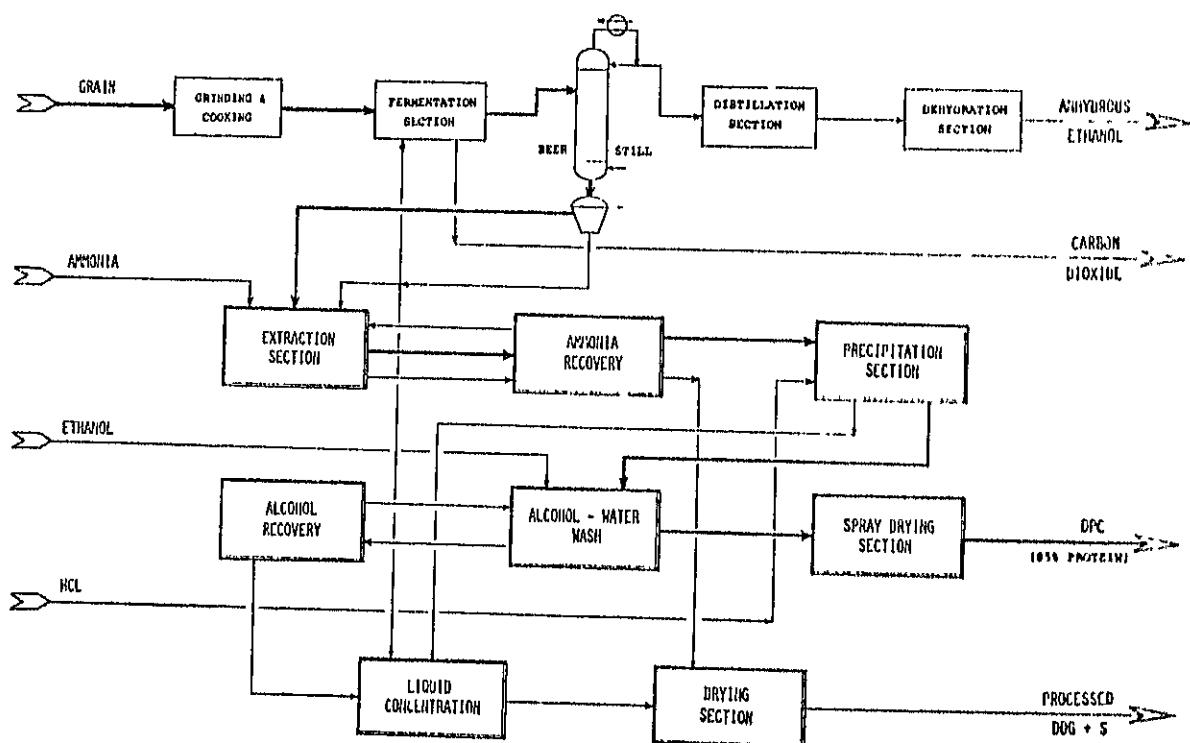


FIGURE 2 - Protein Recovery Process (Ammonia Case).

fed to additional distillation facilities where purity is increased to 95% and to an extractive distillation section where additional water is removed, yielding a 200 proof or anhydrous ethanol. Such dehydration is normally not encountered in the beverage industry but would be found in some plants producing ethanol for industrial purposes. From the bottom of the beer still, we produce a mixture of solid materials and alcohol free liquid, known as stillage. The stillage is centrifuged yielding a liquid stream which under normal circumstances would be partially recycled to the fermentation section for pH control while the remainder would go to a liquid concentration section where the solids content would be increased to about 50% and then mixed with the solids from the centrifuge and dried to yield distiller's dried grains plus solubles.

In the case of protein extraction, the solids from the centrifuge are fed to the extraction section of the plant where the protein is extracted with a caustic solution. Because any additional water added to the system eventually must be evaporated at a considerable expense, we use a portion of the thin stillage to form our caustic solution. Extract from the extraction section is fed to the precipitation section of the plant where hydrochloric acid is added to adjust the pH to the isoelectric point for the protein causing precipitation of the protein concentrate. This protein concentrate is sent to an alcohol and water washing section of the plant where its purity is increased to 85%. The wet protein concentrate is spray dried to yield a distillers protein concentrate (DPC) of 85% purity. Liquid from the precipitation part of the plant is sent to the liquid concentration section. Alcohol from the wash section of the plant is sent to an alcohol recovery still and the alcohol free liquid from this section of the plant is also sent to the liquid concentration section. Solids from the extraction section of the plant are sent to the drying section where they are mixed with the concentrated liquid solution and dried in a fluidized drying and conveying operation to yield processed distiller's dried grains plus solubles. This product contains about 18% protein.

An economic evaluation for this process indicated that there was considerable expense involved in the use of caustic for pH control. When the caustic reacts with the hydrochloric acid, sodium chloride results and this cannot be economically regenerated to permit the reduction in the net caustic usage. Another material was sought for pH control. After consideration, ammonia appeared to meet our requirements since it has been shown to be usable for protein extraction (4) and it is a gas which can be readily separated and concentrated from water solutions.

Figure 2 is the process flow diagram for a conventional grain alcohol fermentation plant that is equipped for protein recovery, this time using ammonia for pH control, rather than caustic. The flow diagram arrangement for the grain fermentation

and alcohol purification is the same as before. The distiller's grains from the centrifuge go to the extraction section of the plant and thin stillage is used to produce an ammonia solution of the proper pH. The extract from the extraction section is sent to an ammonia recovery section where ammonia is stripped from the liquid solution and recycled to the extraction section. The extract is then sent to the precipitation section of the plant where the process continues in the same fashion as before. The solids from the extraction section of the plant are also sent to an ammonia recovery section, where the ammonia is removed by distillation and also recycled to the extraction section. The stripped solids are sent to the drying section of the plant.

TABLE I  
ECONOMIC COMPARISON OF CAUSTIC  
AND AMMONIA EXTRACTION PROCESSES

Ethanol Plant Capacity	20,000,000 gal/yr.	
Increased Investment for NH <sub>3</sub> Case	\$414,000	
<u>Incremental Cost</u>	Caustic Case	Ammonia Case
Chemicals	\$752,000/yr	BASE
Utilities	BASE	\$361,000/yr
Labor	BASE	40,000
Maintenance	BASE	21,000
TOTAL	\$752,000	\$422,000
Incremental Profit for NH <sub>3</sub> Case	\$330,000/yr	
Less Incremental Depreciation (10%)	-41,400	
Less Incremental Taxes (50%)	<u>-144,300</u>	
Incremental Net Profit for NH <sub>3</sub> Case	<u>\$144,300/yr</u>	
Return on Incremental Investment $\frac{144,300}{414,000} \times 100 = \underline{\underline{34.9\%}}$		

Table I contains an economic comparison of the two extraction processes. Based on a 20 million gallons per year ethanol plant, the ammonia extraction case requires the investment of an additional \$414,000 for equipment over the caustic extraction case. However, we see that the caustic case consumes \$752,000 per year more chemicals than does the ammonia case. On the other hand, the utilities, labor and maintenance requirements in the ammonia case are \$422,000 greater than the caustic case. The difference in these two cases indicates that the ammonia extraction case would produce an incremental profit of \$330,000 per year more than the caustic extraction case. Depreciation on the additional equipment required in the ammonia case would be \$41,400 and in all

of our economic studies we have assumed that the federal and state corporate tax rates would total 50%. This leaves a net incremental profit for the ammonia extraction case of \$144,300 per year or a return of 34.9% on the incremental investment of \$414,000. On this basis, further discussion is limited to the ammonia extraction case as shown in Figure 2.

TABLE II  
FEED AND PRODUCT  
QUANTITIES FOR ALCOHOL PRODUCTION

<u>Without Protein Recovery</u>	<u>Corn</u>	<u>Milo</u>	<u>Wheat</u>
Grain, bu/CD (1)	20,920	21,490	21,570
Ethanol, gal/yr	20,000,000	20,000,000	20,000,000
DDG+S, tons/CD (3)	194	210	261
CO <sub>2</sub> , tons/CD	174	174	174
 <u>With Protein Recovery</u>			
Grain, bu/CD (1)	20,920	21,490	21,570
Ethanol, gal/yr	20,000,000	20,000,000	20,000,000
DPC, lb/yr (2)	20,900,000	23,720,000	24,220,000
DDG+S, tons/CD (3)	162	174	225
CO <sub>2</sub> , tons/CD	174	174	174

Notes:

1. Corn and Milo: 56 lb/bu, 15.5% moisture  
Wheat: 60 lb/bu, 14.0% moisture
2. 85% Protein
3. 10% Moisture

Economic studies and market studies, indicate that an economically sized fermentation alcohol plant for present construction should have a capacity in the order of 20 million gallons per year of anhydrous ethanol. Table II contains feed requirements and product output for such a plant when using corn, milo or wheat as a grain source. We see that about 20,900 to 21,600 bushels per day of grain are required to produce 20 million gallons per year of anhydrous ethanol. These grains yield between 194 and 261 tons per day of distiller's dried grains plus solubles. They also produce CO<sub>2</sub> as a by-product. If protein recovery is added to the processing we see that a distiller's protein concentrate containing 85% protein could be produced in the amount of 20,900,000 lbs. per year to 24,200,000 lbs. per year. The quantity of distiller's dried grains plus solubles is somewhat reduced as a result of having removed the protein. The CO<sub>2</sub> production remains the same.

TABLE III  
 INCREMENTAL RETURN ON INVESTMENT  
 FOR PROTEIN RECOVERY FROM  
 DISTILLERS DRIED GRAINS AND SOLUBLES  
 (Feed ratio Wheat:Corn:Milo = 1:1:1)

<u>Material</u>	<u>Conventional Fermentation</u>	<u>With Protein Recovery</u>
Grain, bu/CD	21,330	21,330
Ethanol, gal/yr	20,000,000	20,000,000
DPC, lb/yr	---	22,950,000
DDG+S, tons/CD	222	187
CO <sub>2</sub> , tons/CD	174	174
Incremental Plant Investment:	\$7,600,000	
Incremental Conversion Cost:	\$3,160,000/yr	
Incremental Income: DPC, 45¢/lb DDG+S, \$110/ton / \$83/ton	\$10,330,000/yr -3,250,000/yr	
	\$ 7,080,000/yr	
Incremental Profit	\$3,920,000/yr	
Less Depreciation (10% St Line)	760,000/yr	
Less Taxes (50%)	1,580,000/yr	
Net Incremental Profit	\$1,580,000/yr	
Return on Incremental Investment:	$\frac{1,580,000}{7,600,000} \times 100 = 20.8\%$	
Incremental Net Cash Flow:	\$2,340,000/yr	

If an alcohol plant were to feed a mixture of equal parts of corn, milo and wheat, the grain requirement would be 21,330 bushels per calendar day as shown in Table III. Such a feed would produce 22,950,000 pounds per year of distiller's protein concentrate and 187 tons per calendar day of processed distiller's dried grains plus solubles containing about 18.5% protein. Without protein extraction, we would produce 222 tons per day of distiller's dried grains plus solubles containing 30.4% protein. The incremental plant investment necessary to add protein recovery facilities to a conventional grain fermentation plant is estimated to be \$7,600,000 based on prices of January 1, 1975. The incremental conversion cost for producing the protein concentrate is \$3,160,000 per year. The incremental income for the plant would be \$10,330,000 from the DPC priced at 45¢ per pound, but there would be a reduction in income from the sale of distiller's dried grains plus solubles in the amount \$3,250,000 giving a net incremental income of \$7,080,000 per year. The incremental profit associated with the protein recovery is \$3,920,000 per year. After the deduction of depreciation and taxes, a net profit of \$1,580,000 per year results. This represents a return on the incremental investment of 20.8%. The incremental net cash flow

from the protein recovery is \$2,340,000 per year.

In the evaluation contained in Table III we used a price of 45¢ per pound of DPC. Since no market for this product now exists it is difficult to say exactly what its value is. We do, however, feel that this figure of 45¢ per pound does have a consistent relationship to sodium caseinate priced at \$1.00 per pound, Promine D priced at 60¢ per pound and Amoco's Torutein (yeast) at 42¢ per pound.

Table IV contains information on the amino acid content, protein efficiency ratio, and protein content of corn and wheat along with these quantities for associated DPC's. Comparable quantities for casein are included for reference. The percent protein recovered from the distiller's grains is also shown in the table.

TABLE IV  
PROTEIN COMPOSITIONS, RECOVERIES  
AND EFFICIENCY RATIOS

	WHEAT (1)		CORN (1)		CASEIN
	Grain	DPC	Grain	DPC	
%Protein Recovered	--	36.4	--	54.0	--
%Protein	13.9	87.9	7.8	85.0	90.0
PER	1.44	1.20	1.44	1.08	2.50
Protein:	Gms/100 gms protein				
Isoleucine	3.1	3.2	4.8	3.9	5.5
Leucine	5.8	9.6	10.9	12.0	8.1
Lysine	2.8	3.1	2.9	3.5	7.2
Methionine	0.7	2.2	0.4	1.7	2.6
Phenylalanine	3.5	4.1	5.0	5.2	4.5
Threonine	2.8	3.7	3.7	2.7	4.2
Tryptophan	--	1.6	--	0.7	1.2
Valine	4.4	5.1	4.6	5.6	6.4
%Nucleic Acids	--	1.6	--	1.0	--

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1. Information from the final report on the University of Nebraska NSF Grant No. AER74-10456 A01 entitled "The Development of a High Protein Isolate from Selected Distillers By-Products," July 1975.

## CONCLUSIONS

The results of these studies on the recovery of a protein concentrate from distiller's grains indicate that such recovery has an attractive return on investment associated with it. This return is dependent on the price of distiller's protein concentrate, which has yet to be established through functional studies and market tests. The use of ammonia for pH control in the extraction process appears to have distinct economic advantages with an associated attractive rate of return. At the present time, studies are underway in the process laboratories in the Department of Chemical Engineering at the University of Nebraska to confirm the suitability of ammonia in stillage as an extraction material. Laboratory studies of the functional and nutritional properties of distiller's protein concentrate are underway in the Department of Food Science and Technology at the University.

The potential exists for recovering more than 100 million pounds per year of distiller's protein concentrate from by-products of the distilling industry. The residual distiller's grains are still suitable as cattle feed and thus can contribute to further protein production. It is the opinion of the authors that with continued research the distiller's protein concentrate can become a valuable component in fighting world nutritional problems.

## LITERATURE CITED

1. U.S. Treasury Dept., "Alcohol, Tobacco and Firearms, Summary Statistics," Pub. ATF P 1323.1 (4-74), Fiscal Year 1973. pp. 7
2. Private Communication with Dr. Lawrence E. Carpenter, Distillers Feed Research Council, Cincinnati, Ohio. July 11, 1975.
3. University of Nebraska, "The Development of a High Protein Isolate from Selected Distillers By-Products," Final Report on NSF Grant No. AER74-10456 A01, July 1975, pp. 68-105.
4. Saunders, R.M. et. al., "Preparation and Properties of Protein Concentrates by Wet-Processing Wheat Mill Feeds," Proc. 8th National Conference on Wheat Utilization Research, ARS W-19, Sept. 1974, pp. 88-92.

NUTRITIVE ASSESSMENT AND POTENTIAL FOOD APPLICATIONS OF PROTEIN CONCENTRATES PREPARED BY ALKALINE EXTRACTION OF WHEAT MILLFEEDS

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Introduction. When wheat is milled for flour in the conventional manner, the residue (termed millrun) left after removal of the flour is either used as is or further fractionated into components such as shorts, bran, germ, red dog and middlings. All of these fractions are generally termed millfeeds. These millfeeds are rich in protein, fats, vitamins and minerals (1), but their high fiber content, bitter flavor, and susceptibility to rancidity precludes their widespread use in human foods. Approximately 5 million tons of millfeeds, containing about 800,000 tons of protein, are produced annually in the United States, and are used almost entirely in ruminant feeds. As protein shortages become more acute, it may become advantageous if millfeed protein was fed to humans more efficiently. This could be achieved by direct consumption of millfeed protein, or by increased use in poultry feeds. This becomes more important when it is realized that millfeed protein is of higher quality than flour protein (2), and that the majority of fat, vitamins and minerals naturally reside in the millfeed fraction of the kernel.

Wheat protein concentrate (WPC) which is derived by dry milling of wheat bran or shorts has been used extensively in wheat product shipments in donation programs (e.g. Flour Blend A and wheat-soy blend, WSB). A different wheat protein concentrate (referred to here as wet alkaline process wheat protein concentrate) has been derived from millfeeds by wet alkaline extraction, though only on an experimental basis (3, 4). This concentrate differs from dry milled WPC in that it contains more protein and fat, and less fiber. It is anticipated that it would find different food applications. This paper describes some nutritional and functional properties of those protein concentrates prepared by alkaline extraction of wheat millrun.

Preparation of Wet Alkaline Process Wheat Protein Concentrate<sup>a/</sup>. A flow sheet illustrating the preparation of concentrates from millfeeds is shown in Figure 1. The millfeed is mixed with five volumes of dilute alkali, and gently agitated at room temperature for 15 minutes at pH 8.6-9.0. From the aqueous phase which is separated from the fibrous residue by centrifugation or pressing, the extracted protein is isolated as follows. In Process I, the pH is lowered to 4 whereby a product is precipitated (referred to as acid precipitated concentrate), or alternatively, the pH is lowered to 6 and steam is injected to attain a final temperature of 85°C; the precipitated material is referred to as heat precipitated concentrate. In Process II, the separated aqueous phase is first centrifuged to remove most of the starch as a separate product. The supernatant is then treated as in Process I to produce acid and heat precipitated products. This paper will describe data measured only on those concentrates isolated from unfractionated millrun using Process II (i.e. materials which are low in starch).

a/ Referred to as WAP-WPC.

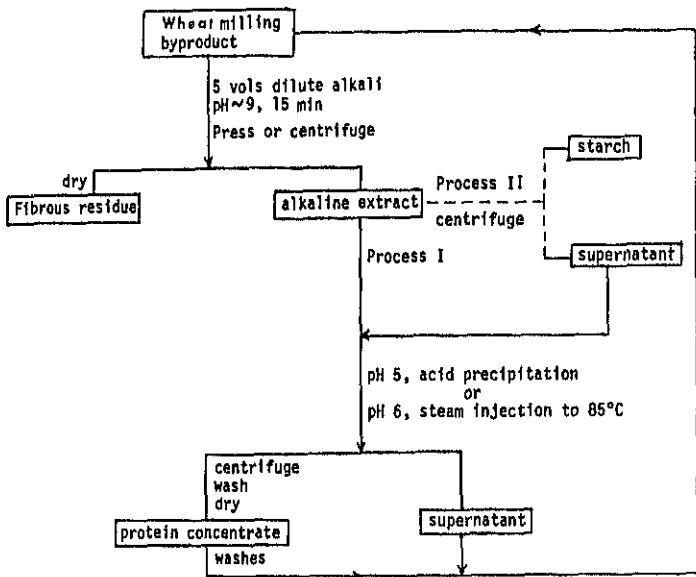


Figure 1. Flow chart of the continuous batch extraction process used in the preparation of protein concentrates by wet alkaline extraction of wheat millrun.

The concentrates isolated by acid or heat precipitation were either freeze-, spray-, or drum-dried. Freeze-dried materials were used as controls to assess possible nutritional changes brought about by high temperature drying. Freeze-drying was done at 25°C in a modified Stokes vacuum oven. Spray-drying was accomplished using a Bowen dryer, with inlet and outlet temperatures of 232°C and 107-112°C, respectively. A steam-heated double drum dryer (Buflovak) with a drum inlet temperature of 127-132°C was used for drum-drying. The samples were ground through a 20-mesh screen prior to evaluation.

Composition. The composition of protein concentrates prepared from millrun by alkaline extraction in the pilot plant are listed in Table 1. The composition of the original millrun is also shown. In the heat-precipitated concentrates, materials dried at high temperature as compared to the freeze-dried sample had apparent low crude fat values measured by ether extraction. However, total lipids measured by chloroform-methanol (2:1, v/v) extraction were similar. Chloroform-methanol extraction of acid precipitated materials was not carried out, although similar results would be expected. These data are included to emphasize the potential error of relying on crude fat values determined by ether extraction in materials dried at higher temperatures. The protein content of the concentrates, normally 60-65%, is increased to about 80% if the lipid is removed.

Table 1. Composition<sup>1/</sup> of protein concentrates prepared by alkaline extraction of wheat millrun<sup>2/</sup>

Precipitation and Drying Method	Protein (N x 6.25)	Crude Fat <sup>3/</sup>	Total Lipid <sup>4/</sup>	Fiber	Ash	Starch
- - - - - Percent - - - - -						
Acid freeze-dried	60.8	13.0	-	0.5	3.9	6.5
spray-dried	60.8	7.9	-	0.5	4.1	8.1
drum-dried	59.2	8.4	-	0.4	4.1	7.4
Heat freeze-dried	58.7	16.1	22.2	0.4	6.0	4.7
spray-dried	57.3	11.6	20.6	0.5	5.3	5.5
drum-dried	56.1	10.7	21.5	0.4	5.3	5.5

<sup>1/</sup> Moisture-free basis.

<sup>2/</sup> Wheat millrun: 22.8% protein, 7.5% fat, 9.8% fiber, 5.5% ash.

<sup>3/</sup> Ether extraction.

<sup>4/</sup> Chloroform-methanol (2:1, v/v) extraction.

Protein Nutritional Quality and Digestibility. The essential amino acid composition (determined by the method of Kohler and Palter (5)) and chemical score of flour, millrun, and of the heat precipitated concentrates dried by three different methods are shown in Table 2.

Table 2. Essential amino acid composition and chemical score of wet alkaline process wheat protein concentrate, wheat millrun and flour

Amino Acid	1973 FAO Provisional Scoring Pattern	Heat Precipitated Concentrate			Millrun	Flour
		Freeze Dried	Spray Dried	Drum Dried		
- - - - - (g amino acid/16 g N) - - - - -						
Lysine	5.4	4.5	4.4	4.9	4.1	1.9
Isoleucine	4.0	4.2	4.0	4.2	3.6	4.1
Valine	5.0	6.3	5.9	5.6	5.1	4.5
Leucine	7.0	7.5	7.4	7.6	6.5	6.8
Threonine	4.0	3.9	3.9	4.1	4.3	2.6
Methionine + cystine	3.5	4.3	4.2	4.2	4.2	4.5
Phenylalanine + tyrosine	6.1	8.1	7.8	8.1	7.1	7.9
Chemical Score	100	83	81	91	76	35

With the exception of lysine which is slightly limiting, the other essential amino acids are present in quantities equivalent to the FAO Provisional amino acid pattern. The chemical score of the concentrates was more than twice that of flour. In a standard Protein Efficiency Ratio (PER) bioassay with weanling rats, values for the concentrates were more than double that obtained for flour protein (Table 3). The extracted protein, that is, the concentrate protein, was more digestible than millrun protein.

Table 3. Protein Efficiency Ratio and nitrogen digestibility of wet alkaline process wheat protein concentrate, wheat millrun and flour

Material	PER	Nitrogen Digestibility <sup>1/</sup> %
Wheat millrun	1.86	73
Wheat flour	0.73	93
Casein	2.50	100
Concentrate, heat precipitated, freeze dried	2.34	93
Concentrate, heat precipitated, spray dried	1.92	92

<sup>1/</sup> Corrected for metabolic N excretion.

Vitamins and Minerals. Values determined<sup>b/</sup> for iron, thiamine and riboflavin are summarized in Table 4 and are compared with equivalent literature values for flour. The iron content was higher in the heat than in the acid precipitate. Thiamine was most preserved in the freeze-dried samples.

Fatty Acid Composition. The major fatty acids were identified as linoleic (54%), oleic (23%), palmitic (20%) and linolenic (3%), or, 80% unsaturated. Storage studies, and in particular the changes in fatty acid composition during storage, have been described in detail elsewhere (6). In general, the WAP-WPC is most stable when stored at very low moisture content.

Potential Food Applications. Protein isolates and concentrates find their way into the market place as a result of their nutritional and functional properties. Highly nutritious, non-functional proteins and, conversely, functional but nutritionally inferior proteins have limited applications in food products.

b/ Carried out by P. M. Ranum, Pennwalt Corp.

Table 4. Iron, thiamine and riboflavin content<sup>1/</sup> of wet alkaline process wheat protein concentrate, and wheat flour<sup>2/</sup> (mg/lb)

Precipitation and Drying Method	Iron	Thiamine	Riboflavin
Acid	freeze-dried	166	3.9
	spray-dried	170	3.4
Heat	freeze-dried	250	3.8
	spray-dried	241	2.8
	drum-dried	246	3.2
Flour	1.9	0.7	0.2

1/ Moisture-free basis.

2/ Literature value for Hard Red Spring wheat flour (Ref. 7).

The functional properties are those properties which impart desirable characteristics to various food systems. Functional properties may be studied by either (1) incorporating the protein directly into foods as a replacement for one or more ingredients, or (2) evaluating various functional properties of the protein using a variety of methods and techniques. The limitations of (1) are time, cost, and quantity of protein required, whereas (2) is limited by the predictive accuracy of the method used.

Functional properties of WAP-WPC such as the Protein Dispersibility Index (PDI), nitrogen solubility, whipping capacity and stability, fat absorption capacity, and baking quality have been studied.

Protein Dispersibility Index (PDI). This property provides an indication of how well the protein would remain dispersed if it were part of a beverage or other system which were rapidly blended and consumed shortly thereafter. The standard A.O.C.S. procedure was used (8). This essentially measures nitrogen remaining in suspension after 20 g of WAP-WPC in 50 ml of water have been blended at 8500 rpm for 10 min, and centrifuged at 2700 rpm (920 x G) for 10 min. Although the acid precipitates were somewhat more dispersible than the heat precipitates (compared with a PDI of 38% for soy isolate), WAP-WPC was not highly dispersible (Table 5). Invariably the order of dispersibility was FD > SD > DD.

Nitrogen Solubility. The solubility of a protein is a function of many factors including its configuration, amino acid composition, ionic strength of the solvent and the extent of denaturation which may have occurred. Solubility would be a desirable property if beverages, soups, or fluid-based products were to be fortified. Nitrogen solubility was determined according to a method described by one of the authors in an earlier study (9). This method involved slowly mixing (120 rpm) 100 mg of the protein concentrate in a final volume of 10 ml of distilled water, adjusting and maintaining the pH at values of from 2 to 10 for 1 hr, centrifuging at 4000 x G for 20 min,

Table 5. Protein Dispersibility Index of wet alkaline process wheat protein concentrate

Method of Precipitation	pH of Dispersion	Method of Drying <sup>1/</sup>		
		Freeze Dried	Spray Dried	Drum Dried
Acid	6.0 - 6.1	12.8 ± 0.5	11.3 ± 0.4	9.0 ± 0.3
	7.0 - 7.2	21.3 ± 0.8	18.9 ± 0.7	13.7 ± 0.6
Heat	5.8 - 6.0	8.3 ± 0.3	7.7 ± 0.2	6.2 ± 0.1
	7.1 - 7.2	11.6 ± 0.3	11.0 ± 0.3	8.1 ± 0.2

<sup>1/</sup> Mean ± standard deviation.

filtering, and determining the percent nitrogen remaining in the supernatant. Since >95% of the N of WAP-WPC is insoluble in trichloroacetic acid (10), N solubility is indicative of protein solubility.

As ionic strength of the solvent (contributed by NaCl) was increased, solubility of the freeze dried, acid precipitated WAP-WPC decreased at pH 10 (from 55% to 40%) (Figure 2). Thus, foods with high concentrations of salt

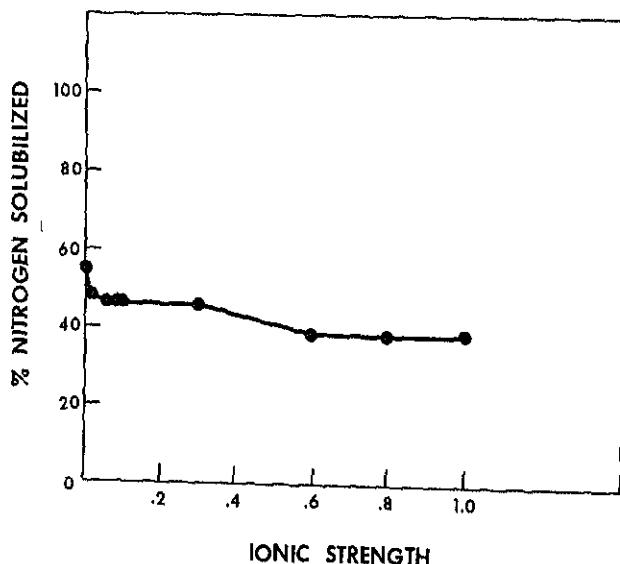


Figure 2. The influence of ionic strength (NaCl) on the solubility at pH 10 of freeze dried, acid precipitated protein concentrates from wheat millrun.

would tend to diminish the solubility of WAP-WPC. The N solubility of freeze dried WAP-WPC was only slightly influenced by increasing the concentration of WAP-WPC from 1 to 10%. Increasing the temperature of the solvent to 70-90°C increased the N solubility of freeze dried, acid precipitated WAP-WPC more than 50% (10). This implies that WAP-WPC is not heat sensitive at these temperatures after processing.

The method of precipitation (acid or heat) and drying had the most profound effect upon N solubility of WAP-WPC. Preparations isolated by heat were never more than 30% soluble at any pH value between 2 and 10 (Figure 3).

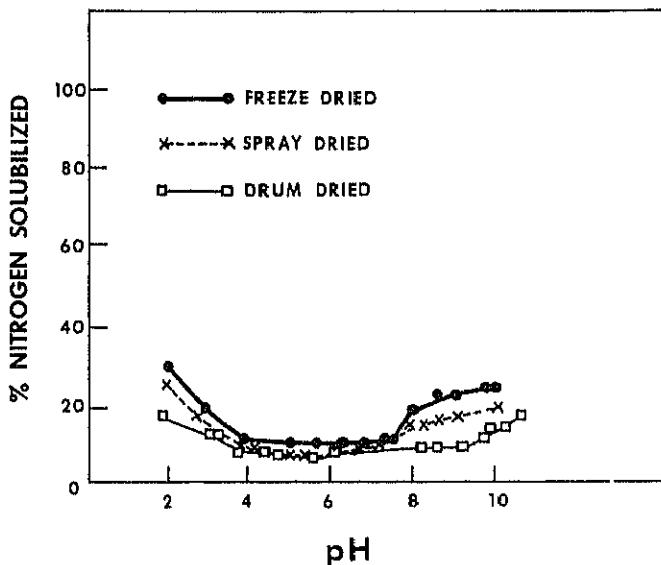


Figure 3. Solubility profiles of heat precipitated protein concentrates from wheat millrun.

The acid precipitates were more soluble with the freeze dried concentrates being most soluble, e.g., nearly 60% was soluble at pH 2 and >50% soluble at pH 10 (Figure 4). The % N solubilized, at pH values other than the isoelectric point, of the freeze, spray and drum dried WAP-WPC was inversely correlated with temperatures attained during the drying process. In general, the N solubility of WAP-WPC is moderate as a function of pH.

Foaming Capacity and Stability. The ability of proteins to incorporate air and form stable foams has been attributed to surface denaturation of the protein and a decrease in surface tension. Foaming capacity and stability would be useful in baked products such as sponge or chiffon cakes and in shipped desserts and toppings. Foaming capacity was determined

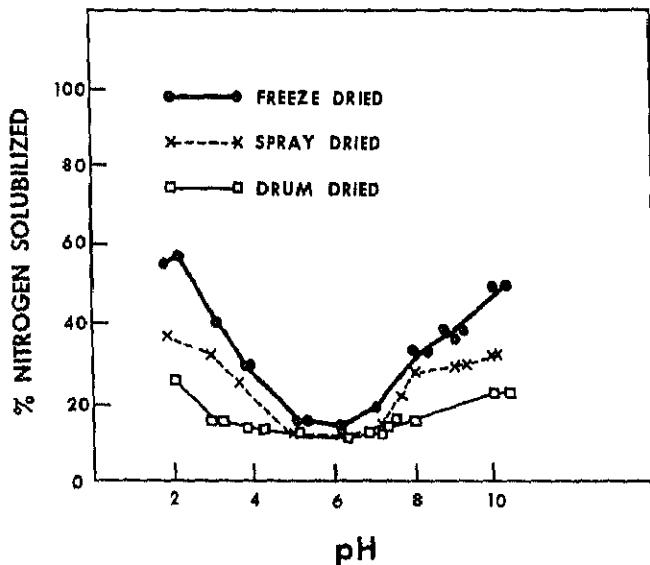


Figure 4. Solubility profiles of acid precipitated protein concentrates from wheat millrun.

according to the method of Lawhon et al. (11) with some modifications. Five g WAP-WPC were added to 100 ml of distilled water at 25°C, dispersed, mixed at speed 7 of a Hamilton Beach mixer for 6 min, transferred to a graduate cylinder and foaming capacity was measured as the actual percent volume increase. Foam stability was subsequently recorded as foam volume as a function of time. The spray dried WAP-WPC exhibited the best foam capacity after whipping (Figure 5). All of the acid precipitated WAP-WPC produced foam volumes which compared favorably with soy protein isolate and concentrate. Of equal, if not greater significance, is the stability of the foam. The spray dried, acid precipitate was the only foam which was stable for more than 5 min (Figure 6). If this were set with heat during baking it may have some potential in food systems. Temperatures of 85°C used to isolate the heat precipitated WAP-WPC impaired the foam stability markedly.

Fat Absorption Capacity. This property is useful in preventing loss of excess fat during food preparation. The method used was a modification of that described by Lin et al. (12). The freeze dried WAP-WPC performed best and was equivalent or superior to soy protein isolate (Table 6). The spray and drum dried preparations absorbed slightly less than the soy isolate but more than the soy concentrate. This property would be useful in preventing the loss of fat during preparation of a meat-protein patty and produce a juicy product.

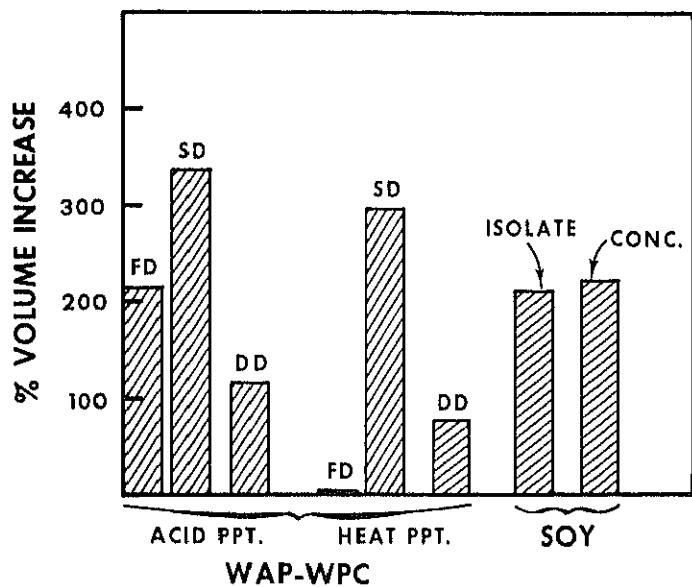


Figure 5. Increase in volume of 5% (w/v) dispersions of various wet alkaline process wheat protein concentrates after whipping.

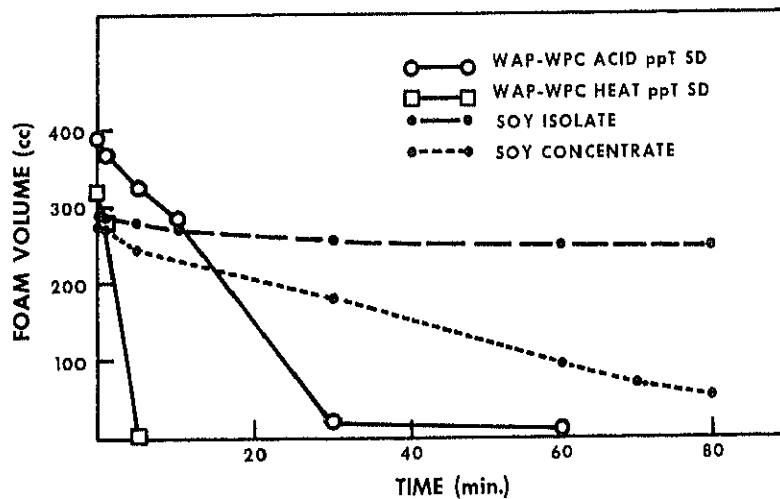


Figure 6. Foam stability of whipped wet alkaline protein wheat protein concentrates.

Table 6. Fat absorption capacity of wet alkaline process wheat protein concentrate

Sample	% Fat Absorption <sup>1/</sup>
Acid precipitate	
freeze-dried	152 ± 6.4
spray-dried	133 ± 6.4
Heat precipitate	
freeze-dried	179 ± 6.4
spray-dried	137 ± 0
drum-dried	137 ± 0
Soy protein concentrate	95 ± 5.2
Soy protein isolate	147 ± 9.9

<sup>1/</sup> Mean ± standard deviation.

Baking Quality. Laboratory pup loaves and one-pound, commercial-sized loaves were baked supplemented with 10 to 20% WAP-WPC as replacement for the hard red winter wheat flour (13, 14). The straight dough process used was patterned after that used in government purchase specifications for soy-fortified flours (15). The baking performance of WAP-WPC in both types of loaves, with 3% shortening (Crisco) as the dough improver, was similar. An example of the subjective evaluation of laboratory pup loaves is shown in Table 7. In general, the acid precipitated WAP-WPC performed better than those which were heat precipitated with the drum dried preparations producing the best loaves (Figure 7).

The protein content of one-pound loaves, baked by the American Institute of Baking, was increased from 14.1% ( $N \times 5.7$ ) for the control to 18.5 to 23.3% for loaves containing 10 to 20% drum dried WAP-WPC. The quality of protein in these supplemented breads was evaluated by amino acid analyses (5), nitrogen digestibility and Protein Efficiency Ratio. The quantity of the limiting amino acid, lysine, increased from 1.79 g/16 g N in the control to 2.83 to 3.30 g/16 g N in breads supplemented with 10 to 20% WAP-WPC (14).

Evaluation of these supplemented breads in a rat bioassay revealed that N digestibility was invariably ≥ 90%, thus, being utilized efficiently by the rat. The PER of various breads containing WAP-WPC ranged from 1.3 to 1.7 compared to 0.76 for the control (Table 8), representing an increase in PER of from 70 to 120% over the control. Duncan's Multiple Range Test (16) showed that the PER of supplemented breads was not significantly different as a function of method of precipitation (acid or heat), or washing procedure. Drying method used did influence PER with breads containing drum dried WAP-WPC having significantly higher PER than the unwashed samples which were spray dried (Table 8). Although the loaves containing 10 to 20% drum dried WAP-WPC

Table 7. Baking quality of wet alkaline process wheat protein concentrate

Sample	Specific Loaf Volume (cc/g)	Break and Shred (5)	Grain (15)	Texture (15)
Wheat flour	5.63	3.25	12.0	13.0
10% Soy flour	5.02	2.75	13.0	13.0
10% Wet alkaline process wheat protein concentrate				
Acid precipitate				
freeze-dried	4.56	2.50	13.0	12.0
spray-dried	4.92	2.50	12.5	13.0
drum-dried	5.26	3.00	13.0	13.0
Heat precipitate				
freeze-dried	3.04	0	10.0	9.0
spray-dried	4.41	2.00	12.0	12.0
drum-dried	5.17	3.00	13.0	13.3

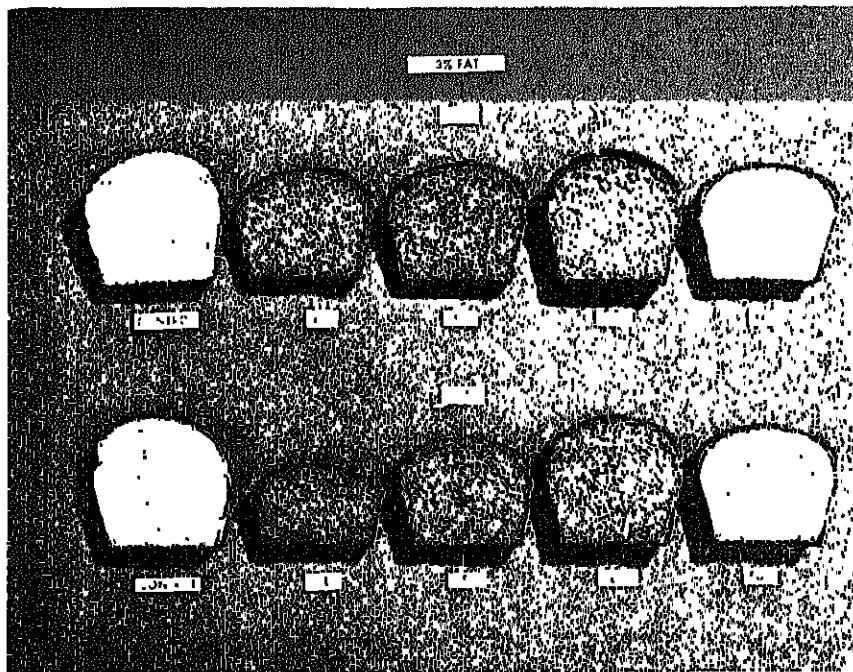


Figure 7. Wheat flour breads supplemented with 10% wet alkaline process wheat protein concentrate or 10% soy flour. Acid and heat represent method of precipitation; FD, SD and DD signify freeze, spray or drum drying.

Table 8. Protein Efficiency Ratio and nitrogen digestibility of breads supplemented with 10% wet alkaline process wheat protein concentrate

Sample	PER <sup>1/</sup>	Nitrogen Digestibility <sup>2/</sup>
Casein	2.50 <sup>A</sup>	100.6
Wheat flour control	0.76 <sup>E</sup>	96.4
10% Soy flour	1.73 <sup>B</sup>	92.4
10% WAP-WPC from millrun <sup>3/</sup>		
Acid precipitate		
spray-dried, washed	1.52 <sup>BCD</sup>	91.6
spray-dried	1.27 <sup>D</sup>	92.4
Heat precipitate		
spray-dried	1.37 <sup>CD</sup>	93.2
drum-dried	1.71 <sup>B</sup>	90.4

1/ Duncan's Multiple Range Test; means without a superscript letter in common are significantly different. P =  $\alpha$  0.05.

2/ N Digestibility =  $\frac{N \text{ Intake} - (\text{Fecal N} - \text{Endogenous Fecal N})}{N \text{ Intake}} \times 100$

3/ Wet alkaline process wheat protein concentrates; samples unwashed unless otherwise indicated.

had a similar PER, the PER of those containing 20% was significantly lower than those with 10%. Since the lysine content of breads with 20% WAP-WPC was higher, it is not, apparently, as available. This serves to emphasize the vital role which bioassays play in evaluating the protein quality of baked products, especially those which have been supplemented with protein and amino acids.

End Use of Process By-Products. In addition to protein concentrates, the other products from the process described are a fibrous residue, a solubles fraction, and a high-starch fraction, if this is separately removed during the process. The fibrous residue, containing approximately 15% protein and 15% fiber, could be used as a feed ingredient in rations for lactating cows. The solubles fraction contains about 17% protein and 40% sugars on a dry basis. Conceivably this fraction could be combined with the fibrous residue prior to drying, or might find use as either a liquid feed supplement ingredient, or a growth medium for cellular organisms. The starch fraction, if removed as a separate product, normally contains 80 to 90% starch but can be further purified if desired.

Applicability to Wet Milling Processes. The recovery of alkali solubilized protein from millfeed fractions could possibly be extended to include protein recovery from whole wheat as well. Exploratory runs using ground whole wheat have been made (17). In recent years the Pillsbury Hydro process (18) and the Far-Mar-Co wet fractionation process (18, 19) have been developed

for the wet milling of whole wheat to produce a variety of products including starch, vital gluten, germ and bran fractions. Although the details of these processes have not been fully described, they may be adaptable to recovery of an alkali solubilized protein concentrate as well.

Economics. A detailed economic study of the process in any of its variations has yet to be completed. A major factor in the overall economics of producing protein concentrates by a wet processing technique will be the cost of drying the final products. Because of strict controls on the amount of BOD, COD and soluble solids allowed in discharge streams to local water treatment systems, it is likely that make-up water or wash water containing soluble solids from the millfeed will have to be evaporated in the process or otherwise treated prior to discharge. It is, therefore, advantageous to recycle the spent liquor or supernatant and use it as the extractant for additional millfeed, thus reducing the amount of water required for the process. On a laboratory scale, this has been shown to be feasible (4). Recycling would improve the economy of operation, thus making it more competitive with the production of protein concentrates from other sources.

In Summary. This paper has described the wet alkaline process, the composition and yields of the protein concentrates, some of the nutritional and functional properties of these preparations and suggested uses for by-products. It has presented a feasible, alternative use of the nutritious by-products of the flour milling industry.

#### Literature Cited

1. MacMasters, M. M., Bradbury, D., and Hinton, J. J. C. Microscopic structure and composition of the wheat kernel. In Wheat: Chemistry and Technology (2nd ed.), ed. by Y. Pomeranz; p. 51. Amer. Assoc. of Cereal Chem., St. Paul, Minn. (1971).
2. Miladi, S., Hegsted, D. M., Saunders, R. M., and Kohler, G. O. The relative nutritive value, amino acid content, and digestibility of the proteins of wheat mill fractions. Cereal Chem. 49: 119 (1972).
3. Saunders, R. M., Eetschart, A. A., and Kohler, G. O. By-products utilization. Preparation of cereal protein concentrates. Baker's Digest 49: 49 (1975).
4. Saunders, R. M., Connor, M. A., Edwards, R. H., and Kohler, G. O. Preparation of protein concentrates from wheat shorts and wheat millrun by a wet alkaline process. Cereal Chem. 52: 93 (1975).
5. Kohler, G. O. and Palter, R. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44: 512 (1967).
6. Betschart, A. A., Saunders, R. M., Mon, T. R., and Kohler, G. O. Variations in the fatty acid composition of stored wheat protein concentrates prepared by wet and dry milling. Cereal Chem. 52: 439 (1975).

7. Millfeed Manual. Millers' National Federation, Chicago, Illinois (1972).
8. A.O.C.S. "Official and Tentative Methods," Tentative Method Ba 10-65, third ed. American Oil Chemists Society, Champaign, Ill. (1972).
9. Betschart, A. A. Nitrogen solubility of alfalfa protein concentrate as influenced by various factors. *J. Food Sci.* 39: 1110 (1974).
10. Betschart, A. A., Saunders, R. M., Bickoff, E. M., and Kohler, G. O. Solubility profiles of protein concentrates. *Cereal Science Today* 18: 276 (Abstract #138) (1973).
11. Lawhon, J. T., Cater, C. M., and Mattil, K. F. A whippable extract from glandless cottonseed flour. *J. Food Sci.* 37: 317 (1972).
12. Lin, M. J. Y., Humbert, E. S., and Sosulski, F. W. Certain functional properties of sunflower products. Presented at the 58th Annual AACC Meeting, St. Louis, Mo., Nov. 4-8, 1973.
13. Betschart, A. A., Saunders, R. M., Bean, M. M., and Kohler, G. O. Effects of processing on the baking quality of wet alkaline process wheat protein concentrate. *Cereal Chem.* 52: (in press) (1975).
14. Betschart, A. A., Saunders, R. M., and Hepburn, F. N. Supplementation of one-pound loaves with wet alkaline process wheat protein concentrates-Baking and nutritional quality. *Cereal Chem.* 53: (submitted for publication) (1976).
15. Agricultural Stabilization and Conservation Service, United States Department of Agriculture, Announcement WF-9, Purchase of soy-fortified bread wheat flour for use in export programs, Sept. 27, 1972 (ASCS Commodity Office, 6400 France Ave. South, Minneapolis, Minn. 55435).
16. Duncan, D. B. Multiple range and multiple F tests. *Biometrics* 11: 1 (1955).
17. Wu, Y. V., Sexson, K. R., and Cluskey, J. E. Wet milling of wheat and oats by alkaline extraction. *Proc. 8th Nat. Conf. on Wheat Utilization Res.*, ARS W-19, pp. 93-96 (1974).
18. Fellers, D. A. Fractionation of wheat into major components. In Industrial Uses of Cereals, Y. Pomeranz, Ch., AACC, St. Paul, p. 212-214 (1973).
19. Henry, W. and Rao, G. V. Whole wheat fractionation process. *Proc. 8th Nat. Conf. on Wheat Utilization Res.*, ARS W-19, pp. 84-87 (1974).

## UTILIZATION OF HIGH-PROTEIN FLOURS IN AID PROGRAMS: ON SITE EVALUATION

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For many years Western Regional Research Laboratory has been involved in developing products and writing quality specifications for products used in U.S. AID Food for Peace programs overseas. Wheat protein concentrate, prepared by dry milling shorts and bran, was developed by our Cereals Laboratory and originally used in Flour Blend A. It was later incorporated as a cooked product into wheat-soy blend (WSB). More recently our group became involved in development of specifications for the soy-fortified flours (SFF) that were based on the Kansas State Blend K product. Two blends were developed, containing 6 or 12% soy flour.

Because of our involvement with these high protein foods, it seemed valuable to observe first hand how the products were being used and what problems were being encountered. Perhaps some could be corrected here in the U.S. at the point of origin. In August, 1975 I visited three countries: Sri Lanka, India, and the Philippines, in that order. I was fortunate to have the assistance of Wheat Associates personnel in helping me set up contacts with U.S. AID, CARE and Catholic Relief Services people.

Sri Lanka (Ceylon). Most of the U.S. donated foods in Sri Lanka are distributed through CARE from their base in Colombo. They make three products from the soy-fortified flour and WSB. For school feeding programs, they make buns for local distribution in a few small operations, and biscuits (cookies) for more extensive distribution throughout the country. For pre-school children, they have developed a product called Thriposha.

Figure 1 shows the plastic bag Thriposha is packed in for distribution. It holds 1 1/2 lbs. of product. The word Thriposha means all three nutrient groups in the native language (Sinhalese). These nutrients were described as protein for body building, carbohydrate for energy and vitamins and minerals for protection. Thriposha now contains 80% WSB and 20% locally-produced sorghum flour. The near-term plan is to substitute local soy flour for 5% of the WSB. This will increase the protein content and also accomplish another step forward toward making the product from indigenous crops and Title I wheat so as to minimize dependence on donated food and hopefully someday reach self-sufficiency.

The Thriposha blend is produced for CARE by the Ceylon Biscuit Company. The sorghum is milled and then baked as a cookie (or biscuit) to precook it. This product is then milled to a fine crumb prior to blending with the WSB. With the help of AID, CARE will soon have a Brady-cooker-extruder for processing the sorghum instead of going through the cookie baking operation. When they introduce the soy, they plan to cook and extrude the soy and sorghum together.



Figure 1. Front view of plastic bag used for Thriposha in Sri Lanka.

Thriposha is distributed throughout the country to mothers for babies and pre-school children. CARE hopes to reach 120,000 children in hospitals, and through Maternal Child Health Centers managed by local health department personnel. During a two day field trip with CARE employees, I visited a thriposha distribution point in a village near Kandy. It was set up in the village council room and the local nurse was in charge. She issued monthly allotments of two bags of Thriposha (1 1/2 lbs. each) per child. This amount supplied about 10 g of protein per day.

During our visit, the CARE Nutritionist, a native Sri Lankan, talked with the mothers about their babies health and the acceptability of the Thriposha. Because she is a native, she can communicate effectively with the mothers in helping them understand the importance of good nutrition and how the CARE products play a role in the health of their children. The effectiveness of this particular product (Thriposha) was revealed in a small way when the nurse in charge showed us a child who had been brought to the center a few months ago as having only skin and bones. Since then, she had gained weight and had flesh on her and was held by a very happy and proud mother. During field trips like this, the CARE people check to see if there are any problems in the distribution system as well as checking the acceptability and effectiveness of their products.

Throughout this trip, I was impressed with the competency of the people in the field. I believe we in the U.S. are lucky to have such people in these programs. These are the people who actually deliver the products we all work so hard to provide. Both the native workers and the Americans who work for the voluntary agencies must be given a major part of the credit for the success of these programs.

The largest CARE program in Sri Lanka is the biscuit program which reaches 800,000 children in school. The biscuits are made by Ceylon Biscuit Company from the 12% soy-fortified flour and WSB. They use four parts SFF and one part WSB. Oil and sugar are also included in the formula and enough water for processing by a rotary cutter. Each biscuit weighs about six grams and supplies slightly less than one gram of protein. Most of the students receive six biscuits; this will be raised to eight next year. Children with severe malnutrition receive nine biscuits per day and also two bags of Thriposha per month. Their mothers are instructed to use it in preparing other foods for that child. The day we visited one school, a boy had brought in some roti, a flat bread his mother had made for him from the Thriposha. The CARE people were pleased to know this--the product was being used effectively.

The biscuits are packed in plastic lined boxes for shipment via railroad car to schools throughout the country. The transportation system imposes a limitation on a program such as this. For example, these biscuits now reach 800,000 children. They would like to reach one million but there just aren't enough railroad cars available to take care of the increase. This limitation along with others imposed by supplies of boxes, plastic and cost of materials keeps the program at its present level. Paper is very scarce in Sri Lanka, so much of it gets recycled--including the bags the donated foods are shipped in. CARE sells these to local bag manufacturers who cut them up into smaller bags. The proceeds CARE receives are used for a variety of projects. We visited one, a bakery built at a school in Colombo. It is one of the few facilities in Sri Lanka where yeast-leavened products are made for the school feeding programs. The buns contain soy-fortified flour and WSB. The bakery is a hand operation throughout--no mixers--not even bowls or dough troughs are used. The flour and WSB are sprinkled on the table; oil, yeast and water on top, then mixed to a dough with the hands. Minimum dough development occurs. After a rest period, buns are formed and after rising, baked in a wood-fired oven. They are gummy textured and heavy by our American standards, but quite acceptable by their standards.

India. Due to lack of time, I wasn't able to visit distribution points in India but did have a chance to discuss programs with CARE and Catholic Relief Services (CRS) personnel in New Delhi and also visit the Modern Bakeries, Delhi Unit.

CARE's program with soy-fortified flour is based on bread, distributed to school and pre-school children in amounts that provide 12 g. protein and 300 calories per day. The programs tend to be located within 100 miles of the cities where they originate due to lack of transportation facilities to reach farther points. They had tried a biscuit program in Bombay but found it to be too expensive.

The CRS group in New Delhi had just received their first shipment of soy-fortified flour while I was there. Modern Bakeries was producing bread for them, making an 800 g. pullman loaf. Each school child in the CRS program receives 100 g. of this bread; an amount which contains 10 g. protein. The bakery personnel were quite enthusiastic about using the soy-fortified flour for producing the bread for the CRS program. In their process, the dough is divided and panned directly from the mixer, essentially a no-time dough. About one hour elapses between the mixer and the oven. The day we toured the plant, they were installing a new horizontal mixer that will mix a dough in four minutes (batch size based on 175 kg. flour). They now have Artofex mixers which take 35 minutes to develop a dough based on 250 kg. flour. With the new mixer they expect to raise production capacity to 8000 loaves per day from 2200 loaves at present.

I heard no negative comments about the soy-fortified flour. It is apparently superior to the local supply. The few negative comments related to insect infestation being greater in high-protein products than in traditional cereal products. India may have more problems in this area than most countries. However, it is recognized that the protein-fortified products do attract more insects than their unfortified counterparts.

Philippines. The Republic of the Philippines has a very large, well-integrated School Nutrition Program. Since 1970, the Philippine government has been working closely with U.S. AID and the voluntary agencies. The government has assigned personnel at every level--national, regional and local--to this program. Nutrition education and food delivery has become an important facet in the teacher's assignment.

Priority for the feeding program is given to schools in economically depressed areas where malnutrition is the greatest. The malnourished children are identified by their weight relative to their age using a chart which reflects international standards for elementary school children grouped in four zones. Filipino children whose weights fall in the "green" and "white" zones are considered to be at or above normal weight for their age and are not included in the school feeding programs. Only those in the "yellow" (under-weight) and "red" (malnutrition) zones are included, and in some situations, only those in the "red" zone. Weight records are kept on all the students, those in the green and white zones as well as those in the yellow and red zones.

In order to increase the nutritional intake of the children in the under-weight and malnourished categories, a nutribun was selected as the major product. It originally contained high levels of milk solids. The soy-fortified flour provided a timely replacement when the milk solids became scarce. The composition of the bun has changed somewhat since the original formula was developed and the size was reduced when they found the children were eating only half the large bun. Originally the bun weighed about 170 grams and supplied 500 calories. Now the bun size is based on 300 calories or 200 calories plus a supplementary food to make up the extra 100 calories. In one location, molasses was used as a filling in a sliced bun to increase the calorie content.

In some areas the buns are baked in commercial establishments or the bakery may be part of a school facility. We visited a pilot bakery at an

Iloilo city school that makes buns for several schools in the district. At this bakery doughs (based on one 50 lbs. sack of SFF) are hand-mixed in a trough. After about one hour they are developed on a dough brake. Figure 2 shows the sticky, underdeveloped dough during the initial passes through the brake. Figure 3 shows the developed dough after about 15 passes. The day we visited, buns were being made with molasses instead of sugar, a substitution made last year when sugar prices became so high.



Figure 2. Hand mixed dough in initial stage of development through a dough brake.

In another school nutrition program in this same province, at the town of Dumangas, noodles are made from the soy-fortified flour. They are called "Nutridles", coined from the words, nutritious noodles. The nutridles supply 100 calories in a dish containing vegetables and broth. The process for making them is similar to that used in Japan and China. The dough is developed by sheeting several times, then cut and hung to dry. The day we were there the rack of noodles was moved outside to dry in the sunshine. The vice-governor of this Province told me how important this noodle machine was to the community in addition to its use for the school feeding programs. A few years ago when

they had a bad rice crop, they made noodles to supply the community with a suitable rice substitute. Because these people eat rice three times a day as a hot food, the noodles provided a more acceptable substitute than bread.

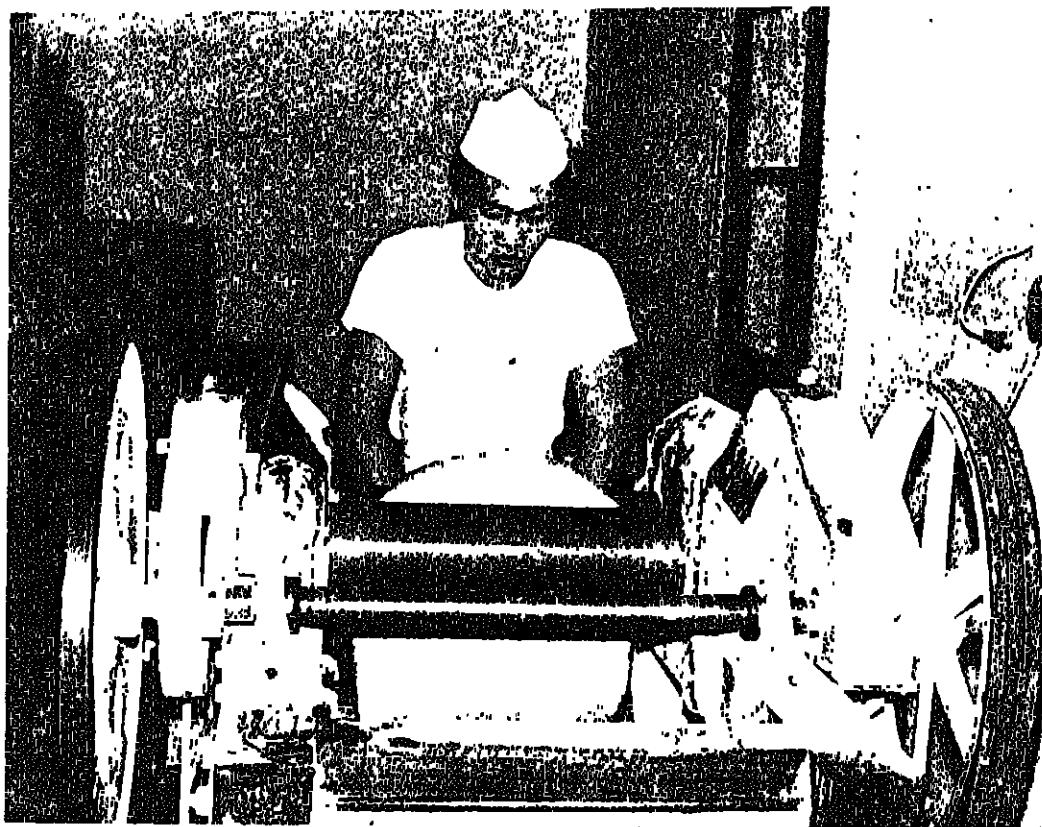


Figure 3. Dough from Figure 2 after development through a dough brake.

Problems Encountered. Because of our laboratory's involvement in developing specifications for the WSB and soy-fortified flour, I tended to look at problems related to those requirements--particularly with regard to the functionality of the soy-fortified flour. There is no doubt we are sending a high quality product. The baking test imposes a criterion (loaf volume) that demands a relatively strong flour and a very effective dough conditioner. It makes very good bread by American standards. From the products I saw overseas, it also has the ability to carry large amounts of WSB in biscuits or buns. Mixing procedures differ greatly for the yeast-raised product and gluten is developed to a lesser extent than in this country. Because of this, I wondered if such a strong flour was necessary. On the other hand, if there are many operations like the one in Sri Lanka that use WSB and soy-fortified flour together in yeast-leavened products, then perhaps the bread flour component should be strong. Additional information and experiments are needed to determine this.

Much of the work at our laboratory was related to storage stability of the soy-fortified flour and showed the improvement brought about by lowering the moisture content of the blend. Such a change could have a major beneficial effect in areas where the temperature in storage facilities varies between 90-100°F. Most products are received quarterly so storage within the country is typically between three and four months. This is in addition to the two-four months between production in the U.S. and delivery in the recipient country. One baker told me they have had trouble with the buns rising if the flour is too old. This agreed with our studies that showed functional deterioration in terms of loaf volume during six months storage at 100°F.

A lower-moisture product would retard such deterioration but would probably add to the cost of the product due to increased processing to achieve the low moisture. An alternative method of improving functional stability is by the use of other more stable dough conditioners. Some are commercially available for other uses but are not yet on the Codex Alimentarius list. This list serves as a guideline for food additives for international programs.

Of course, any stability improvement depends on the container the product is packed in. The present insect-resistant bags seem to be quite effective both toward insects and for preventing moisture uptakes in humid areas. Moisture checks at the receiving end showed no change from the original product moisture when the bags are received in good condition, and most of them are. These bags have four layers of heavy paper and one inner layer of plastic.

The one problem that I saw related to the plastic inner bag and particularly to the way it was seamed longitudinally. A tube of plastic is preferred for these bags but sometimes flat plastic is heat-sealed or stitched to form the tube. The stitched seam causes a problem because it sometimes tears during rough handling after packing, thus negating the moisture-proof character of the bag.

A problem that occurs at the dough mixing stage occasionally is one related to short-weights in bags. As I understand it, the soy-fortified flour does not always pack down easily and bag weights can be off. An adjustment can be made in the contract at the U.S. end to pay for the actual weight of flour received, but this does not help the baker or noodle maker whose batch size is based on one bag of flour. He doesn't have a scale and if the product is 10-20% off-weight, his absorption is off. This happens only seldom, but for him it is a problem.

Another problem I was alerted to was the possibility of rope spores present in the flour. It is hard to imagine these might have been introduced in the United States at the time of packing. Further testing is necessary to determine the source. I was shown some nutribuns that had a pink cast in the crumb indicating some type of contamination, but not necessarily rope. How this contamination occurred and how it relates to the rope spore contamination in the flour is still under investigation. The pink color may well be a problem in the bakery or from some other ingredient.

Summary. The U.S. commitment in donation-feeding programs overseas is based on helping to feed hungry people in critical situations with the ultimate goal of helping them to reach self-sufficiency. The time table for this is short in some countries, but much longer in others depending on economics, politics and mother nature. Within each country, there surely is a desire to take care of its own. For some this is more easily attainable than for others but efforts are being made with the help of our voluntary agencies, supported by U.S. AID. From what I saw, I could cite several examples. The Thripoli project in Sri Lanka uses the donated food as a base, extending it with local foods, sorghum and soy, and ultimately hopes to substitute Title I for Title foods.

In the Philippines, the commitment by that country is larger and more advanced. They are ready to experiment in many directions to maximize their own input. For example, the substitution of molasses for sugar was not at as it might seem to us here. In the Philippines, molasses is not considered human food--only a feed ingredient. Thus, much effort had to be expended to convince people in and out of government that this product had merit. The results justified this effort. The children like the molasses bun better than the sugar bun. The money that is saved on sugar could then be used to support other ingredients or equipment.

In another experiment, milk from the Carabao (Water Buffalo) is being served to some children along with the nutribun. The Philippine government hopes to develop a dairy industry around this traditional beast of burden.

These programs and several others I saw all suggested a desire by the people to help themselves. The U.S. donated products provide a helping hand along the way. I believe everyone in the U.S. involved in the production of these commodities, (farmers, industries, universities and the government), all be proud of them. They are of good quality--and from what I saw--they appreciated overseas. I saw only a few situations in a few countries but I feel the same story can probably be repeated about many places throughout the world of the lesser developed countries.

## A SUGAR-FREE FORMULA FOR REGULAR AND HIGH-PROTEIN BREADS

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### Introduction

The importance and interest in the sugar-free formula for high-protein breads are evidenced by the following: Through September 15, 36 known newspapers having a combined circulation of 7,500,000 have published articles about the bread. In addition, articles have appeared in at least 20 trade journals. There has been television coverage by 5 stations and there were 50 telephone interviews with radio stations, commercial bakers, magazine publishers, milling and food companies, research organizations, etc. Articles have appeared in two Administrator's Letters, in four Deputy's Letters, and in the Congressional Record--Proceedings and Debates of the 93rd Congress, 2nd Session. The information has been published by invitation in Baker's Digest (1) as a review article on "Sugars in Breadmaking." Additionally, about 1300 requests for the formula from homemakers and about 300 from commercial bakers and allied research organizations have been answered.

This paper presents chronologically the several phases of research that led to the production of high-protein breads, using a sugar-free formula, a straight dough, and a 70-minute fermentation time.

### High-Protein Breads with a Sugar Formula

Research published in the late 1960's led to a public USDA patent by Pomeranz and Finney (2) on a process for adding high levels of protein supplements to bread that met with consumer acceptance regarding loaf volume, crumb grain, and freshness retention.

In studies with 4 percent milk solids or 12 percent soy flour in the formula, Finney and Shogren (3) found that sodium stearoyl-2-lactylate (SSL), a recognized conditioner for yeast-leavened sweet goods, produced better crumb grain and somewhat higher loaf volume than twice as much calcium stearoyl-2-lactylate (CSL), a recognized bread-dough conditioner. When 12 percent soy flour was included in the formula, SSL was similar to glycolipids in that a few tenths of one percent produced loaf volumes and crumb grains that were greatly superior to those for 3 percent shortening.

### Studies with Sucrose and Barley Malt

A second phase of research on cereal malts in breadmaking, published in 1972 (4), was the key to producing bread with a sugar-free formula. For example, in straight-dough, 180-minute fermentation time bakes without malt (figure 1, open circles and open triangles), loaf volume increased sharply as sucrose levels were increased from 0 to 2 percent, decreased somewhat at 3 percent sucrose, and increased significantly with further increases in sucrose, for both the milk (NFMS) and no-milk formulas. In the formulations with optimum levels of malt and various levels of sucrose (figure 1, solid circles and solid triangles), the malt requirement decreased as the sucrose level was

increased above 4 percent, for both the milk and no-milk formulas. Increasing sucrose levels from 0 to 6 percent, in the presence of optimum malt, did not increase loaf volume. Loaves baked without added sucrose but with optimum malt were equal in loaf volume to those baked with 6 percent sucrose and optimum malt, and higher than those baked with high levels of sucrose but no malt. Thus, suitable amounts of cereal malt, in place of 6 percent sugar, hydrolyzed enough starch into fermentable sugars to support fermentation during breadmaking.

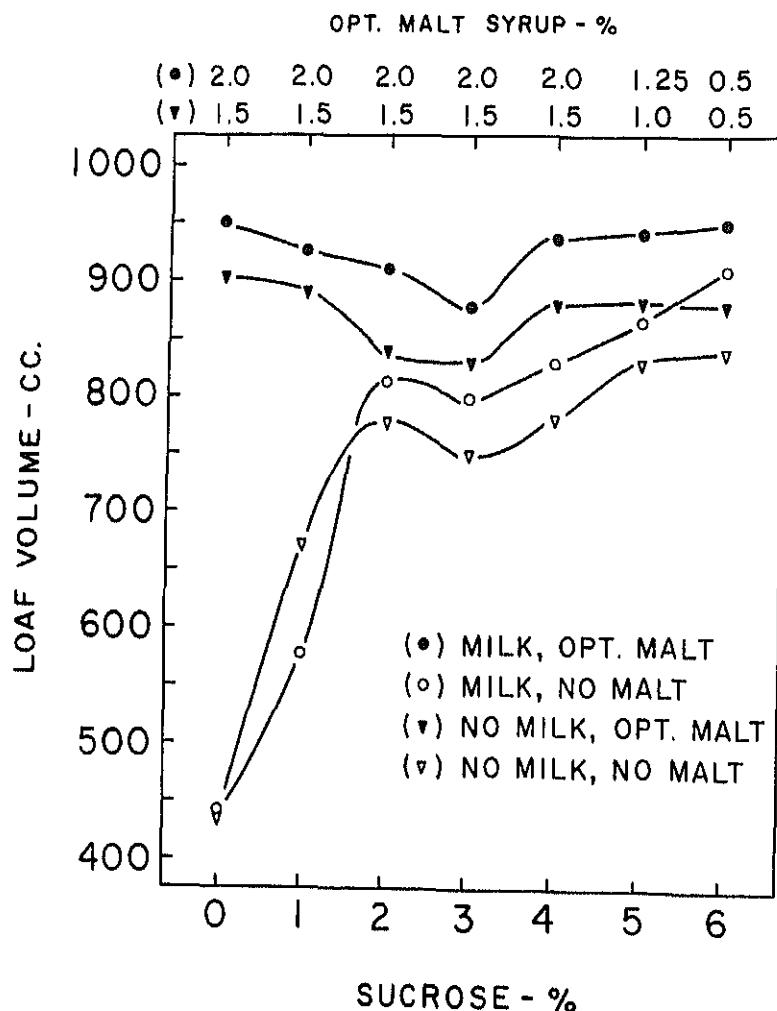


Figure 1.--Loaf volumes of bread baked from 100 grams flour with 0 to 6 grams sucrose by four formulations, including added NFMS with and without optimum malt and no added NFMS with and without optimum malt. Formula also included 1.5 grams salt, 3 grams shortening, 2 grams yeast, and optimum potassium bromate (3 milligrams with 4 grams NFMS and 1 milligram without milk).

### Ascorbic Acid Replaces Potassium Bromate

A third phase of simultaneous research (5) showed that ascorbic acid effectively replaced potassium bromate (not approved in many countries) as a dough oxidant and developer. An integration of the three phases of research resulted in producing high-protein bread with a sugar-free formula that is applicable internationally.

### Sugar-Free Formula for High-Protein Breads

The sugar-free formula used in making high-protein breads is given in table 1. As soy flour increased (table 2), with shortening in the formula, loaf volume decreased from 946 cc. (no soy flour) to 833 cc. (12 percent soy flour). With the formulation containing optimum sucrose palmitate, loaf volume was essentially constant through 10 percent soy flour (937 cc.), and decreased only 28 cc. at the 12 percent level. Similarly, loaf crumb grain score (table 2), for the formulation containing shortening, decreased from satisfactory (S) to unsatisfactory (U) at the 12 percent level. For the sucrose palmitate formulation, crumb grains did not become questionably poorer than satisfactory (Q) until the soy flour level was 12 percent. Typical high-protein breads (figure 2) baked with 6 percent sugar and 10 percent soy flour (top left) and with 6 percent sugar, 10 percent soy flour, and 4 percent soy grits (bottom left) have undesirably brown and thick crusts compared to the thin and golden crusts of the corresponding loaves baked with no sugar in the formula (top and bottom right).

Table 1.--Sugar (regular) and sugar-free (high-protein), straight-dough bread formulae, using 180 minutes of fermentation

Bread ingredients	Formula <sup>1/</sup>	
	Regular	High-protein
	<u>Percent</u>	<u>Percent</u>
Wheat flour	100.0	88-96
Soy flour	-	12- 4
NFMS	4.0	-
Sugar <sup>2/</sup>	6.0	-
Lipid <sup>2/</sup>	Sh. 3.0	SP 0.15-0.65
Malt	0.50	2.0
Oxidizer (p.p.m.)	KBrO <sub>3</sub> 20.0	Ascorbic acid 100.0

<sup>1/</sup> Water optimum, salt 1.5 percent, and yeast 2 percent were common to both formulae.

<sup>2/</sup> Sh. and SP are abbreviations for shortening and sucrose palmitate, respectively.

Table 2.--Effect of 4 to 12 percent soy flour on loaf volume and crumb grain of bread, using the sugar-free formula, optimum sucrose palmitate, and 180 minutes of fermentation

Wheat flour/ Soy flour	Loaf volume		Crumb grain <sup>1/</sup>		Optimum sucrose palmitate
	3% Short- ening	Opt. sucrose palmitate	3% Short- ening	Opt. sucrose palmitate	
	<u>cc.</u>	<u>cc.</u>	<u>Percent</u>		
100/0	946	941	S	VS	0.15
96/4	928	945	S	S+	.25
95/5	915	958	S	S+	.30
94/6	910	945	Q-S	S	.35
93/7	915	953	Q-S	S	.40
92/8	915	950	Q	S	.45
91/9	888	945	Q	Q-S	.50
90/10	898	937	Q-U	S	.55
89/11	860	920	Q-U	S	.60
88/12	833	913	U	Q	.65

<sup>1/</sup>VS, S, Q, and U are abbreviations for very satisfactory, satisfactory, questionable, and unsatisfactory, respectively.

Sucrose palmitate is manufactured by two Japanese companies and has been approved for general food use in Japan and for certain food uses in several European countries. It has not yet been cleared by the U.S. Food and Drug Administration. The lack of clearance of sucrose esters in the United States does not warrant excluding them in studies with high-protein breads. Except for the free polar lipids of wheat flour, we have found no surfactant to be as effective a protein interactor as the sucrose esters containing about 70 percent monoester. Presently, satisfactory high-protein bread can be made with the sugar-free formulation by using 3 percent shortening and 0.5 percent sodium stearoyl-2-lactylate (SSL) instead of about 0.5 percent sucrose palmitate.

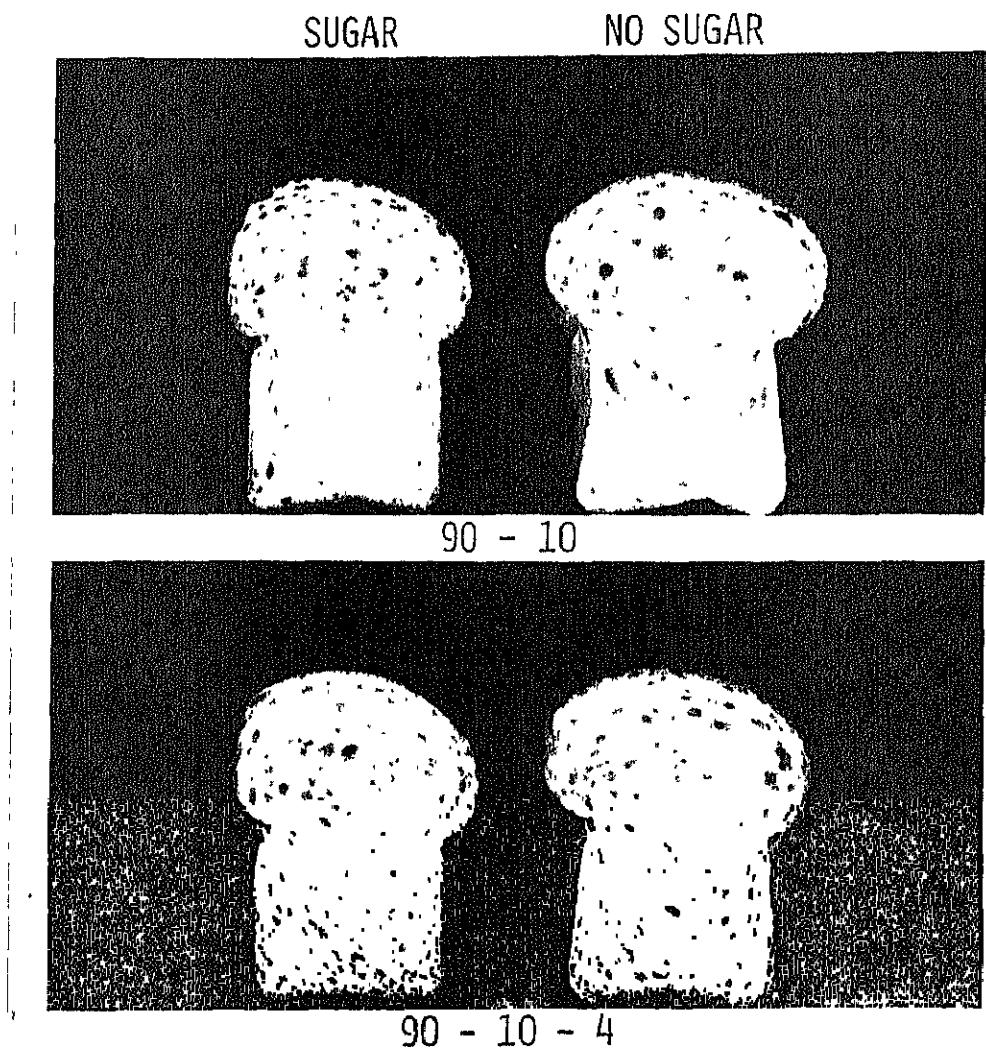


Figure 2.--Typical high-protein breads baked with 6 percent and no sugar in the formula and containing blends of 90 percent wheat flour and 10 percent soy flour, with and without 4 percent added soy grits.

With the sugar-free formula containing 10 percent of soy flour and 4 percent soy grits, bread contains about 13.5 percent protein of high biological value compared to only 9 percent protein in conventional bread, and crusts are golden-brown and thin. Adding high levels of protein supplements, such as soy flour, improves the nutritive value of wheat flour by increasing both protein content and the amount of lysine. Lysine, the limiting amino acid in cereal proteins, is almost tripled. A small amount of dry malt is blended with most breadmaking flours at the mill, but the amount, when sugar is omitted in the formula, is only a fraction of that required to convert (hydrolyze) enough starch of wheat flour to fermentable sugars that support production of carbon dioxide for leavening.

#### A 70-Minute Fermentation Time with Sugar

At the same time the sugar-free formula was being applied to the 180-minute straight-dough method, Finney *et al.* (7) materially reduced production costs by making optimum bread with only a 70-minute fermentation time, instead of 180 minutes, by increasing yeast from 2.0 to 7.2 percent in a straight-dough formula containing sugar. The key to producing optimum bread with a 70-minute fermentation time is centered on optimizing yeast, bromate, and proof time. For example, as fermentation time decreases, yeast concentration increases (figure 3). Plotting (figure 4) KBrO<sub>3</sub> requirements and corresponding fermentation times for 13 flours established the general relation between those two factors. Specifically, each flour's KBrO<sub>3</sub> requirement (relative to 180 minutes) increased by a factor of 1.5 for 120 minutes of fermentation, 3.0 for 70 minutes, and 6.0 for 45 minutes of fermentation, when the corresponding yeast concentrations in figure 3 were used.

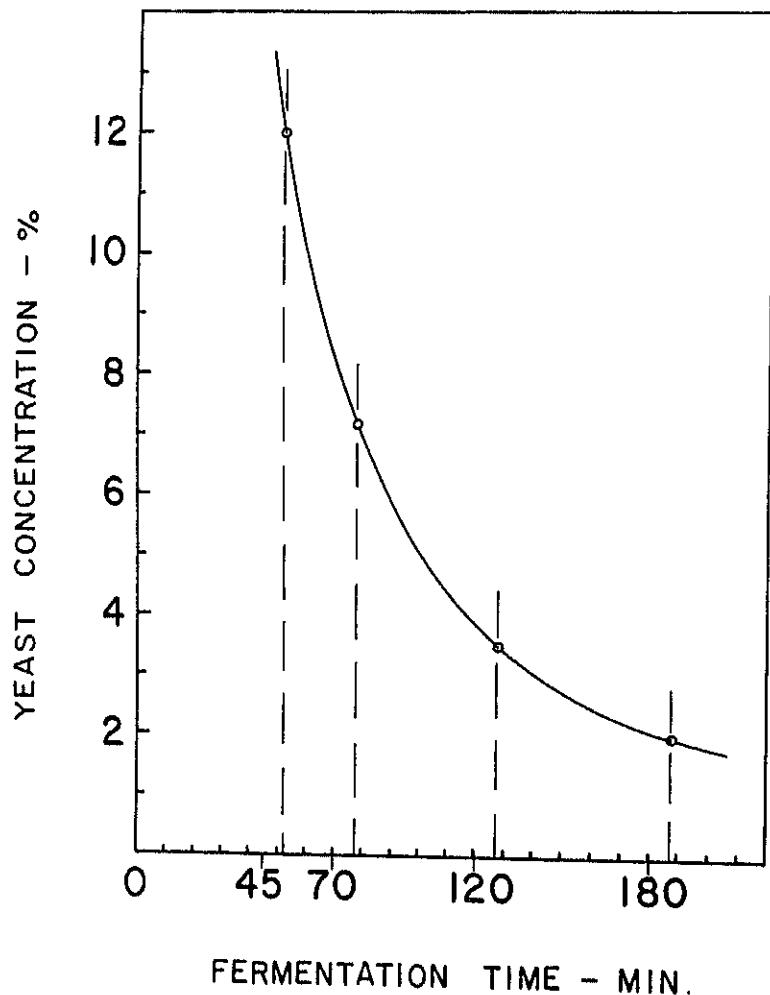


Figure 3.--Yeast concentrations and fermentation times required to produce optimum breads with the standard commercial flour.

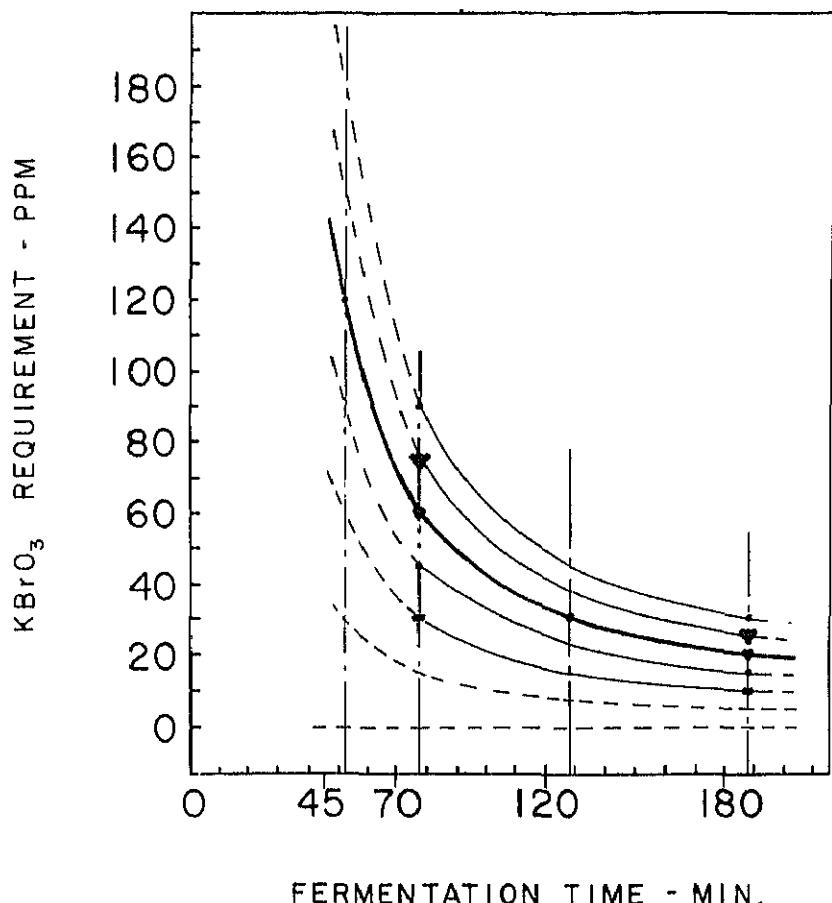


Figure 4.--KBrO<sub>3</sub> requirements and fermentation times required to produce optimum breads for all flours in table 1. See corresponding yeast concentrations in figure 3.

The sugar-free formula is just as applicable to producing regular as high-protein bread (table 1). For regular bread, high levels of protein supplements, such as soy flour and grits, would be eliminated or replaced with 4 to 5 percent of nonfat milk solids (NFMS), milk substitute solids, or soy flour. Soy flour (4 to 5 percent) has properties similar to those of NFMS (6). Also the formula could include 2 to 3 percent shortening and/or 0.25 percent SSL, together with 100 p.p.m. ascorbic acid and about 1.5 percent of the specified barley or wheat malt.

#### Economics of the Sugar-Free Formula (180-Minutes of Fermentation)

It is estimated that 50 million, 1-pound loaves of white pan bread are baked daily in the United States. Omitting 8 percent sugar in the formula, about 3 million pounds of sugar (at 40 cents per pound) or about \$1.2 million could be saved per day. The sugar would be replaced with 540,000 pounds (1.5 percent) of barley or wheat malt. At 20 cents per pound for diastatic malt, the net daily savings would be more than 2 cents per loaf. Probably of equal importance would be reducing the daily sugar requirement by almost 3 million pounds.

Optimum proof times (figure 5) for 120-, 70-, and 45-minute fermentation times were 36.5, 21.5, and 12 minutes, respectively, when the corresponding yeast concentrations in figure 3 and corresponding bromate requirements in figure 4 were employed.

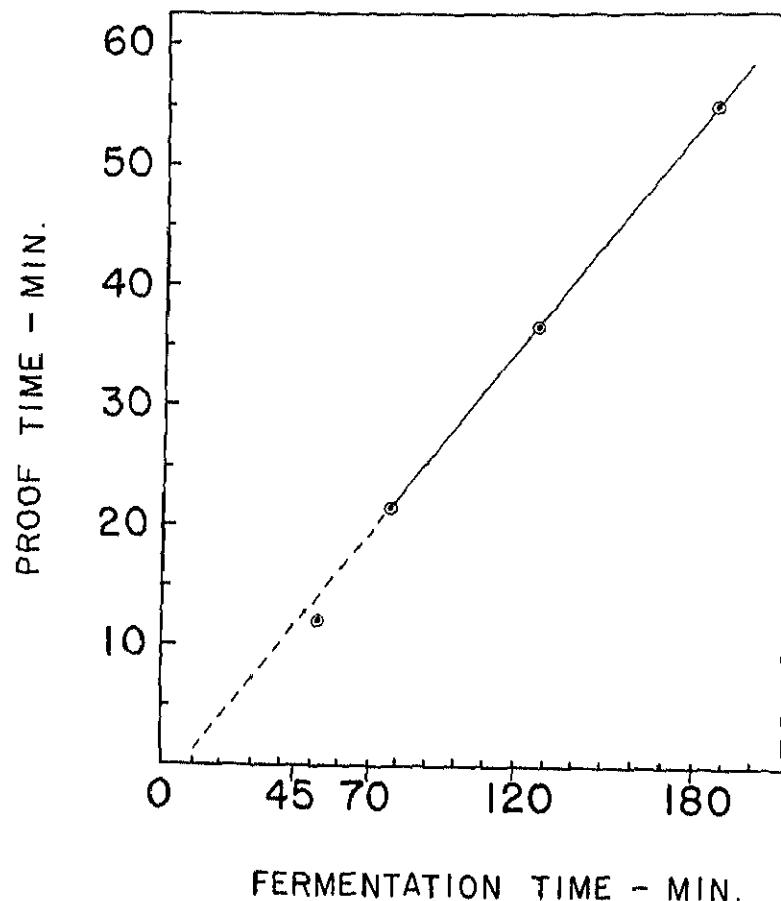


Figure 5.--Proof and fermentation times for optimum bread.  
See corresponding yeast concentrations in figure 3  
and corresponding bromate requirements in figure 4.

A 70-Minute, Sugar-Free, High-Protein Formula

Then Magoffin *et al.* (8) cut the sugar-free breadmaking time in half by combining the short, 70-minute fermentation time and the high-protein sugar-free formula in which 16 percent of wheat flour was replaced with 12 percent soy flour and 4 percent nonfat milk solids (NFMS). In addition, increasing the yeast from 2.0 to 7.2 percent increased about four fold the amount of highly nutritious yeast proteins in bread (table 3).

Table 3.--Sugar-free, high-protein, straight-dough, bread formulae, using 180 and 70 minutes of fermentation<sup>1/</sup>

Bread ingredients	Fermentation (minutes)	
	180 <sup>2/</sup>	70 <sup>3/</sup>
	Percent	Percent
Bakers' flour <sup>4/</sup>	82-100	82-100
Soy flour (Ardex 550)*	14-0	14-0
NFMS	4.0	4.0
Shortening	3.0	3.0
Sodium stearoyl-2-lactylate	0.5	0.5
<i>Yeast</i>	2.0	7.2
<i>Malt</i> <sup>5/</sup> <i>or</i> <sup>6/</sup>		
flour (60 D.U., 20°/g.)	1.5	0.75
syrup (250° L.)	2.0	1.0
KBrO <sub>3</sub> (p.p.m.)	10.0	20.0
Ascorbic acid (p.p.m.)	50.0	50.0

<sup>1/</sup> Water opt. and salt 1.5 percent were common to both formulae. Amounts of malt, oxidizers, and yeast may vary depending on flour properties and treatment, shop conditions, and yeast potency.

<sup>2/</sup> If ascorbic acid is omitted, increase KBrO<sub>3</sub> 5 to 10 p.p.m. If KBrO<sub>3</sub> is omitted, increase ascorbic acid 50 p.p.m. Ascorbic acid usually improves crumb grains.

<sup>3/</sup> KBrO<sub>3</sub> should not be omitted. If ascorbic acid is omitted, KBrO<sub>3</sub> should be increased 40 p.p.m.

<sup>4/</sup> A bakers' flour containing 12.0 to 12.5 percent protein.

<sup>5/</sup> Malt should contain about 60 alpha-amylase units (20° D.U.) per gram or have a Lintner value of about 250°. Amylomalt, a malted barley flour from Ross Industries, Wichita, Ks., was about equal to 250° Lintner malt syrup from Standard Brands Canada Ltd., Montreal, Quebec.

<sup>6/</sup> If bakers' flour is not malted, increase malt 0.25 percent.

\*Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Using the formula containing 90 grams of wheat flour, 10 grams of soy flour, and no NFMS (table 4, 90/10/0), bread had highly satisfactory loaf volume and crumb grain. Those superior loaf characteristics were essentially maintained even when 4 grams of nonfat milk solids were included (86/10/4). However, when the 4 grams of nonfat milk solids were replaced with 4 grams soy flour (86/14/0), loaf volume and crumb grain were distinctly inferior to those for 86/10/4. Thus, 10 to 12 percent soy flour is the practical maximum amount that can be carried by the wheat flour. Replacing 16 grams of wheat flour with 12 grams of soy flour and 4 grams of NFMS (84/12/4) gave a satisfactory (S) crumb grain and loaf volume (900 cc., table 4). Thus, 84 grams of wheat flour carried 16 grams of foreign proteins of high biological value, and the bread contained 50 percent more protein than that made from 100 grams of wheat flour.

Table 4.--Effect of 10 to 14 percent soy flour, with and without NFMS, on loaf volume and crumb grain of bread using the 70-minute sugar-free method (3 percent shortening + 0.50 percent SSL<sup>1/</sup>)

Wheat flour	Soy flour	NFMS	Loaf volume <sup>2/</sup>	Crumb grain <sup>3/</sup>
Percent	Percent	Percent	cc.	
100	0	0	1010 <sup>4/</sup>	VS
90	10	0	980	VS
86	10	4	961	VS
88	12	0	938	S
84	12	4	900	S
86	14	0	875	Q-S
82	14	4	830	Q-S

<sup>1/</sup>SSL is abbreviation for sodium stearoyl-2-lactylate.

<sup>2/</sup>Average proof height was increased from 7.75 to 8.15 cm. for dough containing soy flour.

<sup>3/</sup>VS, S, and Q-S are abbreviations for very satisfactory, satisfactory, and questionable to satisfactory, respectively.

<sup>4/</sup>0.25 percent SSL instead of 0.50 percent.

Typical breads fermented for 70 minutes and containing 100 percent wheat flour (figure 6, top right) and a blend of 86 percent wheat flour, 10 percent soy flour, and 4 percent NFMS (bottom right) have volumes and crumb grains that are somewhat superior to those for corresponding loaves fermented for 180 minutes (top left and bottom left).

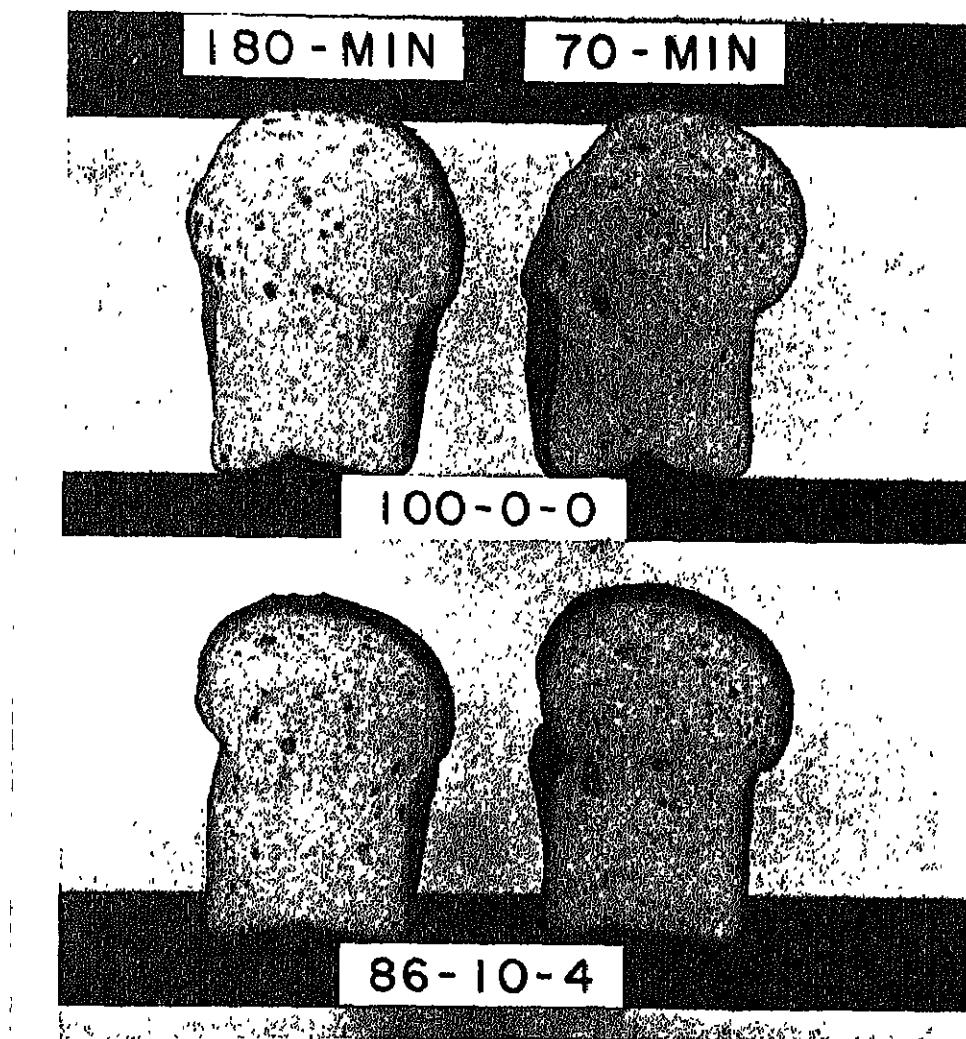


Figure 6.--Typical breads fermented for 70 and 180 minutes and containing 100 percent wheat flour and a blend of 86 percent wheat flour, 10 percent soy flour, and 4 percent NFMS.

### Potential Advantages

The 70-minute fermentation time and the sugar-free formula for high-protein bread has these potential advantages:

- \* Labor costs are reduced, which could lower the cost of bread to consumers.
- \* Net daily savings on ingredients would be at least 1¢ per loaf.
- \* Bread is enriched with 50 percent more protein than supplied in conventional bread.
- \* Amount of the essential amino acid lysine in the protein is tripled, giving amino acid balance and biological value nearly equal to that of meat and milk proteins.
- \* The bread is tastier and has a thin, golden-brown crust.
- \* The sugar requirement of the United States' baking industry could be reduced by 3 million pounds daily.
- \* The sugar imports of the United States could be reduced by 3 million pounds daily, thereby encouraging a lower price per ton.

### Literature Cited

- (1) Pomeranz, Y., and Finney, K. F. Sugars in breadmaking. *Baker's Digest* 49(1): 20. 1975.
- (2) Pomeranz, Y., and Finney, K. F. Protein-enriched baked products and method of making same. United States Patent No. 3,679,433. July 25, 1972.
- (3) Finney, K. F., and Shogren, M. D. Surfactants supplement each other, make foreign proteins compatible in breadmaking. *Baker's Digest* 45(1): 40. 1971.
- (4) Finney, K. F., Shogren, M. D., Pomeranz, Y., and Bolte, L. C. Cereal malts in breadmaking. *Baker's Digest* 46(1): 36. 1972.
- (5) Shogren, M. D., and Finney, K. F. A mixture of ascorbic acid and potassium bromate quickly optimize loaf volume. *Abstracts, Cereal Sci. Today* 19(9): 397. 1974.
- (6) Finney, K. F. Loaf volume potentialities, buffering capacity, and other baking properties of soy flour in blends with spring wheat flour. *Cereal Chem.* 23: 96. 1946.

- (7) Finney, P. L., Magoffin, C. D., Hoseney, R. C., and Finney, K. F. Short-time baking systems. I. Interdependence of yeast concentration, fermentation time, proof time, and oxidation requirement. *Abstracts, Cereal Sci. Today* 19(9): 413. 1974. *Cereal Chem.*
- (8) Magoffin, C. D., Finney, P. L., and Finney, K. F. Short-time baking systems. II. A sugar-free formula for conventional and high-protein breads. *Abstracts, Cereal Foods World* 20(9): 460. 1975.

WHEAT GERMPLASM RESOURCES - THE PROGRAM AT THE  
BELTSVILLE AGRICULTURAL RESEARCH CENTER

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The USDA World Collection of small grain varieties and strains at Beltsville, Maryland, is an invaluable resource for use in current and future research to improve the yield and quality of the wheat crop. This is one of the two largest collections in the world; the other is at the N. I. Vavilov All-Union Institute, USSR. The USDA collection contains more than 70,000 accessions of wheat, barley, oats, rye, and near-relatives of wheat. Wheat is represented by about 31,000 entries.

"There has been much discussion in recent years of the question of the future wheat supply of the world, and in some quarters fears have been expressed that by the end of the next thirty years we may experience a universal wheat famine, provided that the present rate of increase of the bread-eating population and the present yield of wheat per acre shall continue." This is the introductory sentence from a chapter written by Mark Alfred Carleton on "Successful Wheat Growing in Semi-Arid Districts", that appeared in the Yearbook of the Department of Agriculture for 1900.

This was not the first expression of concern over the future prospects of the wheat crop, and it did not prove to be the last. Today, an imposing array of experts have expressed deep concern over the prospects of meeting world food needs (8). Others have underscored the consequences of the loss of irreplaceable genetic resources, the genetic vulnerability of major crop plants, and the critical problems that confront us in increasing or, at the very least, maintaining current yield levels (1, 3, 4).

Carleton devoted much of the 1900 report to the prospects of improving average wheat yields in the Great Plains. He stressed the importance of the Crimean wheat that had been brought to Kansas from Russia some 25 years previously and had, by then, spread into Nebraska, Iowa, and Oklahoma. There seemed little question but that the hardiness of Crimean wheat had revolutionized the winter wheat industry of the middle Plains States. In spite of Mr. Carleton's disdain for the name, Turkey, which he felt was misleading, it was destined to prevail over Crimean. The role of Turkey wheat in the history of the Plains States is commemorated by a monument in Newton, Kansas.

History of World Collection. The early history and expansion of wheat production in the United States can be traced to the use of named varieties and sources introduced from other countries and subsequent selection of productive, adapted plant types from these introductions. The obvious contribution of plant introductions to American agriculture led to the organization in 1897 of the Seed and Plant Introduction Office of the U.S. Department of Agriculture. There has been a continuing effort since that time to: (a) collect and maintain plant material that may contribute either directly or indirectly to crop improvement; (b) establish reliable procedures to preclude the inadvertent introduction of new diseases, insects, and weeds; and (c) distribute useful introductions for use by plant breeders and growers. The value of the world collection of small grains was greatly enhanced by a special appropriation provided under the Research and Marketing Act of 1946. This increased support was used to grow introductions in quarantine, insure the maintenance of viable seed, accumulate data on adaptation and reaction to pests, catalogue other information of use in improving the crop, and fill requests for seed.

Reitz and Ward (7) have summarized the systematic preservation of small grain germplasm that was practiced as an outgrowth of cereal breeding and classification of varieties initiated by the former Office of Cereal Investigations of the U.S. Department of Agriculture. They noted the pioneering work of Mark Alfred Carleton in plant exploration, introduction, and evaluation of wheat, oats, and barley in the period from 1898 to about 1912. From 1913 to 1915, C. R. Ball and C. E. Leighty evaluated wheats and other grains obtained from fair exhibits and test plots planted at State Agricultural Experiment Stations. Samples were collected from farmers' fields in about 1916 by C. R. Ball and J. A. Clark. In 1919, Clark and J. H. Martin obtained seed from growers who reported any wheat crop unidentified as to variety in the first national wheat variety survey. In this manner, a complete collection of domestic varieties was assembled. A large number of varieties and selections were observed during this period, and while many were preserved in the World Collection, a significant number were lost because of inadequate resources and inadequate storage.

Need for Continuity. The maintenance of seed stocks is expensive, but much less expensive than the concerted effort needed to locate specific seed sources that have been lost or discarded from breeding programs. Furthermore, the original genetic source may have disappeared in the country or region from whence it was collected. Changing agricultural practices, including the adoption and widespread use of improved varieties, have depleted the natural store of variation that was once found in or near certain major centers of diversity. In addition to agricultural development, intensive grazing, population pressure, and industrial growth have contributed to the loss of natural

plant populations, including primitive forms and wild relatives that serve as a potential reservoir of diversity (2, 3).

Collections of germplasm fall into two major categories, "working collections" and "conserved stocks" (2). Our wheat germplasm is held in a working collection, and as such we insure that all seed stocks are documented, held in adequate storage, and made available for immediate use. In contrast, conserved stocks represent broad segments of germplasm stored for long-term conservation (at seed conservation centers). Seed in conserved stocks duplicate working collections, but accessions are released only when they are not available from other sources. Seed moves from working collections to long-term storage. In general, national seed storage facilities accept all categories of germplasm, establish precise guidelines for acceptance of seed, and provide optimum or near-optimum temperature and humidity for storage. In the United States, the National Seed Storage Laboratory of the Agricultural Research Service at Fort Collins, Colorado, is the center for long-term seed preservation. Somewhat comparable seed storage programs have been established in Japan and Turkey, the latter, in cooperation with FAO.

The significance of germplasm collections can be appreciated when we consider the vulnerability of wheat varieties and the transient working collections held by individual plant breeders. The development of new races and biotypes of pathogens and insect pests poses a continued threat to varietal stability. As a rule, we can expect modern wheat varieties to change completely every decade. According to Reitz and Briggie (5) some 700 varieties of wheat had been used by U.S. farmers in the period from 1915 to 1966. In the Pacific Northwest, new wheat varieties have had a life expectancy of about five years. Some interesting approaches are being studied in the quest for reduced genetic vulnerability. Nevertheless, it is not realistic to assume that there will be any immediate and dramatic changes in current wheat production practices. We will continue to see the "best" available variety, as measured by pest resistance, yield, quality, and adaptation, planted on large contiguous acreages.

Breeders may discard new accessions after limited evaluation because these accessions show little promise of contributing to their particular breeding objectives. All breeders maintain some kind of a collection based on items in current development programs, as well as varying numbers of other selections and varieties. They are restricted in the number of items that can be retained, by either storage facilities or resources available for maintaining viable seed of all new accessions. Also, transient collections can fluctuate widely with changes in emphasis within breeding programs and with changes in personnel. Plant breeders may make immediate use of the specific character or characters expressed in a new introduction.

Norin 10, introduced from Japan by ARS personnel in 1946, has contributed to the development of most semi-dwarf varieties released since that time, especially in the Pacific Northwest and in Mexico (2). In contrast to the rapid acceptance of Norin 10, we can consider the fate of P.I. 178383, an accession from Turkey collected by J. R. Harlan in 1948. This accession had poor milling quality, susceptibility to leaf rust, weak straw and poor winter hardiness. It proved to be highly resistant to stinking smut (bunt), but was not regarded as having value in any other respect. In 1960, however, stripe rust devasted wheat production in some parts of the Pacific Northwest. P.I. 178383 was evaluated further and found to exhibit good resistance to four races of stripe rust, 35 races of common bunt, and 10+ races of dwarf bunt, all of which occur in the Pacific Northwest. In addition, this accession was found to have usable levels of tolerance to flag smut and to snow mold, and to possess outstanding seedling emergence. Thus, except for its use in breeding for bunt resistance, P.I. 178383 was neglected for a number of years before it was recognized as an outstanding source of multiple-disease resistance (2). This is but one example of the importance of maintaining germplasm collections even though particular items may appear to have few, if any, redeeming characteristics.

Maintenance of the Collection. Assembling and maintaining germplasm received from plant exploration and seed exchanges with research workers are major undertakings. Procedures followed in handling our world collection of small grains illustrate both the complexity of the task and the need for cooperation and good coordination among private, state, regional, national, and international organizations. Accessions include mutations, synthesized species, and lines that represent new or unusual gene recombinations (6). Conversely, the world collection is not used to preserve genetic stocks developed by individual geneticists. Considerable effort must be expended to retain specific genotypes free from changes that result from outcrosses, mutations, and mechanical mixtures. This responsibility must be assumed by geneticists. It cannot be performed in a satisfactory manner with our current staffing.

New accessions are fumigated to kill insects. They are then assigned a Plant Introduction (P.I.) number, and a record prepared of available information as to variety, origin, and any special characteristics. A seven gram portion of the accession is treated with a fungicide and set aside for sowing in a detention nursery at Mesa, Arizona.

Each accession is grown under irrigation at Mesa, Arizona, and observed for evidence of such seed-borne diseases as the smuts and viruses. The nursery is isolated from major grain areas, so that latent plant diseases brought in with imported seed samples can be intercepted without endangering our wheat crop. Contaminated entries

are treated and replanted. Entries are discarded if there is evidence that treatment did not eliminate the diseases.

About 200 grams of seed of each entry grown at Mesa are returned to Beltsville for inclusion in the working collection. Seed is stored at 50°F and 50 percent relative humidity. We divide some of the 200-gram seed samples into sets, normally about 4 to 16 sets of five grams each are packaged at one time. These five-gram samples are distributed without charge to research workers for experimental purposes. Requests may range from a single item to a complete set of the collection (more than 30,000 wheats).

All entries in the world small grain collection, including all new accessions, are deposited at the National Seed Storage Laboratory, Fort Collins, Colorado. At this laboratory, long-term preservation is sought by maintaining seed at 40°F and 35-40 percent relative humidity. An additional safe storage facility for the entire collection is maintained at Aberdeen, Idaho.

Arrangements are made to include materials in observation nurseries and in greenhouse plantings at strategic locations. These tests provide for the accumulation of data on disease and insect reaction and agronomic characteristics. Inclusion of accessions in the International Rust Nursery, coordinated from Beltsville, Maryland, is a typical example of planned evaluations. However, most requests for seed are received from cooperating scientists in the United States and in foreign countries. They secure information for their own records and submit data for inclusion in files maintained as an integral part of the world collection of small grains. Information obtained from the nursery at Mesa, Arizona, special observational nurseries, and plantings established by cooperating public and private wheat breeders covers a broad range of characteristics, but may not be accumulated uniformly over all entries. Our entire wheat collection has been evaluated for reaction to bunt, stem rust, sawfly, and cereal leaf beetle, and for both protein and lysine content.

An automated data system is relied on to store and retrieve the following information on each entry: growth habit and plant growth; color of kernels, straw, chaff, and awns; reaction to diseases and insect pests such as rusts, smuts, viruses, green bug, cereal leaf beetle, Hessian fly, and sawfly; and such nutritional properties as protein, specific amino acids, lipids, and fats. The system is programmed to prepare lists of entries to accompany seed shipments and special listings of all or various portions of the collection that possess certain traits in common. It can be programmed also to provide lists of depleted items that need to be multiplied. A list of high lysine wheat accessions in the World Collection is shown in Table 1.

Table 1.--High lysine wheats in USDA World Collection of small grains  
(Beltsville Agricultural Research Center)

<u>CI/PI No.</u>	<u>Name/Designation</u>	<u>Source/Origin</u>	<u>Growth Habit</u>
13447	Sel. 58	Washington	winter
13449	Selection 9	Washington	winter
94540	199 r/e 34	Iran	winter
112344	Little Tich	England	winter
117018		Australia	facultative
117421		Turkey	spring
119317	Albistan Yazlik	Turkey	spring
121814	Droral	India	spring
121815	Drochen	India	spring
135061		Afghanistan	
135070		Afghanistan	spring
135073		Afghanistan	
137737	Gandum-i-Abi	Iran	
137740	Gandum-i-rasmal	Iran	
157600	Sewan No. 85	Korea	winter
166624	Kizilca 247/5	Turkey	winter
166674	Kizilca 71-a	Turkey	winter
166757	Havidi	Turkey	facultative
166759	Beyaz Bug	Turkey	facultative
166859	Cirpiz	Turkey	spring
166901	Saribasak	Turkey	winter
166916	Yumusak	Turkey	facultative
166921	Sam	Turkey	winter
166946	Digrak	Turkey	spring
166951	Sirtiyumru	Turkey	spring
167455	Akca Bugday	Turkey	spring
167681		Turkey	facultative
167697		Turkey	facultative
173438		Turkey	winter
181325		Afghanistan	spring
181329		Afghanistan	spring
184194	Domaca	Yugoslavia	winter
184250		Yugoslavia	winter
191043	Candeal Basto de Calera	Spain	facultative
220350	Gandum	Afghanistan	winter
220358	Gandum-i-Lalmi	Afghanistan	spring
222670		Iran	winter
222671		Iran	spring
222674		Iran	spring
225221	Graecum Marginatum	Iran	winter
225223		Iran	winter
225232	Meridionale	Iran	winter
225243	Kazvinicum	Iran	winter
234860		Ethiopia	spring
268449	Harlan JR 237	Afghanistan	facultative

During 1963-1972, an average of 150,000 samples of wheat, barley, oats, and rye were distributed annually. The average distribution of wheat amounted to more than 56,000 samples as indicated in Table 2.

Table 2.--Samples of wheat distributed from USDA World Collection of small grains (Beltsville Agricultural Research Center)

<u>Year</u>	<u>Foreign</u>	<u>Domestic</u>	<u>Total</u>
1963	14,208	35,396	49,604
1964	1,092	28,140	29,232
1965	34,538	11,414	45,952
1966	16,276	12,542	28,818
1967	7,174	33,319	40,493
1968	23,292	39,230	62,522
1969	33,906	64,129	98,035
1970	16,788	44,257	61,045
1971	43,707	38,130	81,837
1972	36,136	31,672	67,808

Demands by wheat breeders and geneticists exhaust most of our supply within five years or less. Therefore, entries are multiplied every five years or sooner with at least one-fifth of all entries increased each year at either Mesa, Arizona, or Aberdeen, Idaho.

Nature of Accessions. The total amount of basic genetic information in a collection may be more closely related to the number of cross combinations available within the collection than to the total number of accessions. It is possible that the law of diminishing returns has been attained for many characters. Thus, it is reasonable to expect that most genes in any 1000 new lines may duplicate those already present in the collection. However, the new accessions received each year add new characters and new combinations of characters.

Many of the entries in the small grain collection are highly variable. In the past, mixed barley accessions were separated when first received, and maintained as two or more pure-line populations. Conversely, pure line selection is not practiced within the world collections of wheat and oats. It would be simple to increase the number of wheat entries by deriving several pure lines from each accession. This would be a costly undertaking in terms of increased storage space and maintenance and would not increase the germplasm at our disposal. Although morphologically diverse types could be selected in deriving pure lines, useful germplasm could be lost because of the impossibility of recognizing physiological diversity. Since our objective is to retain as diverse a source of wheat germplasm as possible, then it is advantageous to retain bulk lines in preference to pure lines.

Some investigators have asked us to develop and maintain pure lines of wheat while others have advocated compositing accessions to reduce total numbers. We have held to the concept of preserving a sample of each entry as originally received. Viable seed lots are maintained by using 200 seeds to increase each entry at five year intervals, or sooner, as required by demand. Thus, it is mandatory in evaluation work to either grow accessions so that the behavior of each plant can be expressed fully or to derive pure lines. The wheat breeder must select pure lines that appear to fit the needs of his particular hybridization program.

The growth in the world collection of wheat is shown in Table 3. A large percentage of the entries were obtained from plant exploration and through direct exchanges with domestic and foreign wheat breeders. In addition, a number of public, private, and international organizations have cooperated not only in the conservation of germplasm, but also in the exchange of seed samples. These include, but are not limited to, AID, FAS, FAO, N.I. Vavilov All-Union Institute, Izmir Agricultural Research and Introduction Center, CIMMYT (International Maize and Wheat Improvement Center, Mexico City) and the Ford Foundation.

Table 3.--Wheat accessions in the USDA World Collection of small grains (Beltsville Agricultural Research Center)

Year	<u>New Accessions</u>		<u>Total</u>
	<u>Foreign</u>	<u>Domestic</u>	
1962			18,436
1963	396	112	18,944
1964	513	115	19,572
1965	204	23	19,799
1966	199	20	20,018
1967	620	104	20,742
1968	350	51	21,143
1969	842	341	22,326
1970	1,810	564	24,700
1971	3,971	258	28,929
1972	542	1,898	31,369

The major sources of accessions are Asia, Europe, United States, and Australia and New Zealand, with fewer accessions received from Africa, South America, Central America and Mexico, and Canada. Improved relations with the USSR have led to firm commitments to increase the exchange of germplasm with that country. There is every expectation that this exchange program will add several thousand entries to the existing collection. Also, we have made provision for a "gene bank" to accept bulk seed from  $F_1$  and  $F_2$  plants produced by breeders who

work under diverse environmental conditions. These composite samples consist of heterozygous materials from hundreds of crosses. The success of this activity depends on the cooperation of wheat breeders in contributing their excess F<sub>1</sub> and F<sub>2</sub> seed.

Although many varieties of historical significance were lost prior to 1946 because of improper seed storage, there are at least 34 varieties in the collection that were used extensively in the United States before 1900. These include such important varieties as Turkey, Purplestraw, Red Fife, Marquis, and Defiance.

A substantial amount of information has been accumulated on locations where we have been able to find resistance or tolerance to specific disease and insect pests, and to such environmental variables as low soil pH and associated aluminum toxicity. These data are invaluable in the planned acquisition of new collections. There are occasions, however, where desirable characters are found in unexpected sources or locations. Reitz and Craddock (6) mention that our most resistant materials to the wheat stem sawfly were found in the Iberian Peninsula where the insect had never been reported. Resistant varieties have solid pith rather than hollow straw. Solid pith did not improve straw strength as expected, but did improve insect resistance.

Comments. Breeders will always look first for needed genes within their own material because of the improved adaptation of the populations with which they are working (6). The infusion of new germplasm is essential, however, to meet long term goals for improving quality and yield, and to insure the stability of our wheat crop in the face of pests and environmental hazards.

The USDA World Collection of wheat provides an excellent example of the economy that can be achieved by relying on a single location to maintain diverse germplasm. The collection is typical of the national overview of research needs that we will continue to stress at the Beltsville Agricultural Research Center.

#### Literature Cited

1. Anon. 1972. Genetic vulnerability of major crops. National Academy of Sciences, Washington, D.C.
2. Creech, John L. and Louis P. Reitz. 1971. Plant germplasm now and for tomorrow. Advances in Agronomy 23:1-49.
3. Harlan, Jack R. 1975. Geographic patterns of variation in some cultivated plants. Jour. of Heredity 66:182-191.
4. Hanson, A. A. 1969. Modern concepts in plant breeding. Proc. Int. Seed Test. Assoc. 34:369-384.
5. Reitz, L. P. and L. W. Briggles. 1966. Distribution of the varieties and classes of wheat in the United States in 1964. USDA Bul. 368.
6. Reitz, L. P. and J. C. Craddock. 1969. Diversity of germplasm in small grain cereals. Economic Botany 23:315-323.
7. Reitz, L. P. and D. J. Ward. 1959. The USDA world collection of wheat varieties and strains of interest to geneticists. Proc. of First Intl. Wheat Genetics Symp. 143-150.
8. Wade, Nicholas. 1973. World food situation: Pessimism comes back into vogue. Science 181(4100)634-638.

## OPPORTUNITY TO IMPROVE THE NUTRITIONAL VALUE OF WHEAT THROUGH BREEDING

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Breeding for improved nutritional value is an important component of the cooperative Agricultural Research Service-Nebraska Agricultural Experiment Station wheat improvement program. Research on the genetic manipulation of wheat protein at Nebraska dates to 1954. Two considerations prompted the research. Prior to 1954, there had been little breeding effort to make wheat better nutritionally. Secondly, the hard red winter wheat region of which Nebraska is a part, has experienced for as much as 3 decades, significant depression of the protein content of the wheat produced -- especially in the high plains where little nitrogen fertilizer was used.

The research initially was concerned with increasing the protein content of the wheat grain. In 1966, it was expanded to include modification of the amino acid composition of the wheat protein. Success of the research required answers to numerous questions relating to protein. Our findings as they relate to these questions are discussed.

### How can the nutritional value of wheat be improved?

Wheat is an important source of calories, protein, certain vitamins, and minerals. Improvement of any of these would favorably affect its nutritional value. Because of the importance of wheat as a protein source for people in many parts of the world, and because of serious world protein shortages, the ARS-Nebraska research to improve the nutritional value of wheat has concentrated on improving its protein value.

### How good is ordinary wheat protein?

The protein content of wheat is ordinarily higher than that of rice, corn, and sorghum, the other principal food cereals. Like the other cereals, wheat protein contains insufficient lysine for its full utilization by monogastric animals. The digestibility of wheat protein is excellent. Despite its amino acid imbalance, ordinary wheat protein provides adequate nutrition for adult humans. An adult person who eats enough wheat to satisfy his daily energy requirement will also satisfy his protein requirement as well. Wheat is not an adequate protein source for very young children whose protein requirements are much higher than those of adults.

### How much genetic variation for protein content and amino acid composition has been found in wheat?

The ARS-Nebraska team has systematically analyzed all of the common and durum wheats in the USDA World Collection for protein and lysine. The number exceeds 20,000 wheats. The range in protein values was from 7 to 22 percent. A substantial portion of this variation was subsequently determined to be non-genetic. The genetic component of the total variation probably does not exceed 5 percentage points. Several useful genetic sources for high protein have been identified which are being used in breeding programs. They include:

Atlas 66	Nap Hal
Atlas 50	Hybrid English
Aniversario	So. Dakota 69106
Nebr. Male Fertility Restorer	TJB54/224

Variability for lysine among the wheats in the World Collection ranged from 2.2 to 4.0 percent of the protein. As with protein itself, much of this variation has proven to be non-genetic. Some of it has been demonstrated to be an artifact of protein variation. The genetic component of total lysine variation appears to be no more than 0.5 percentage point. This is less than one-half the increase in lysine needed to bring it into balance with the other essential amino acids. We are extensively utilizing an old Indian variety Nap Hal and C.I.13449, an experimental line from Washington, as genetic sources of high lysine in our breeding program. A recent group of Napalese accessions to the World Collection appear to have above-normal lysine and also will be used extensively for breeding purposes in the future.

Is high grain protein in wheat a stable trait?

It is no more possible to fix the protein in wheat at some predetermined high level by breeding than it is to fix the yield of wheat by breeding. High yielding wheat varieties are not always high yielding. Similarly high protein varieties may not always produce grain with high protein content. Environment, particularly soil fertility, exerts strong influence on protein content.

The important question is whether productive wheat varieties can be developed that produce grain with higher protein content than equally productive ordinary varieties grown in the same environment. Our experience indicates that such varieties are possible.

Responses of the high protein line C.I.14016 and Lancer to nitrogen fertilizer at selected low fertility sites in Nebraska are summarized in Table 1.

Table 1.--Average yield and protein responses of C.I.14016 and Lancer wheat varieties to nitrogen fertilizer at several Nebraska test sites in 1969 and 1970

Nitrogen applied (Lb/A)	Grain yield (Bu/A)		Protein content (%)	
	Lancer	C.I.14016	Lancer	C.I.14016
0	38	38	10.8	12.5
20	44	41	11.2	13.3
40	47	44	11.8	14.0
60	46	45	12.6	14.9
80	46	45	13.2	15.4
100	46	45	13.6	15.8
120	45	46	14.0	16.3

Yield responses of the varieties to N-fertilizer were minimal under the low rainfall test environments but there were strong protein responses in both. The average protein content of each variety was increased by more than 3 percentage points with application of 120 pounds per acre of nitrogen. Note that

C.I.14016 maintained an approximate 2 percentage points protein advantage over Lancer throughout the entire range of N-fertilizer applications.

Further evidence of the effectiveness of the Atlas 66 genes for high protein was obtained from the International Winter Wheat Performance Nursery (IWWPN) (Table 2). At 3 test sites from which mean grain protein ranged from very high (17.8%) to moderately low (12.5%), the high protein varieties Atlas 66, NE67730, and Purdue 28-2-1 consistently produced grain with 2-4 percentage points higher protein content than the other varieties.

Table 2.--Phenotypic expression of the high grain protein trait at sites of the International Winter Wheat Performance Nursery in 1970 at which the mean protein level among varieties was high (Stillwater, Oklahoma), intermediate (Martonvasar, Hungary), and low (Cambridge, England)

Variety	Grain protein content (%) at:		
	Stillwater	Martonvasar	Cambridge
	Oklahoma	Hungary	England
Nursery x	17.8	15.8	12.5
Bezostaya 1	16.5	14.3	12.3
Lancer	16.2	14.6	12.1
Yorkstar	16.0	13.7	11.2
Gaines	16.5	14.1	10.6
Atl 66/Cmn (NE67730)	20.9	18.4	14.2
Purdue 28-2-1 <sup>1</sup>	20.8	20.3	13.7
Atlas 66	20.6	19.4	13.5

<sup>1</sup>High protein line derived from Frondoso.

Do increases in the protein content of wheat affect the amino acid composition of the protein?

Increases in protein content up to approximately 15 percent are associated with decreased lysine per unit protein. Increases in protein above 15 percent produce little, if any, further decrease in lysine per unit protein.

Lysine per unit weight of grain increases with protein content. We have determined that high protein wheat provides more lysine and other essential amino acids per weight of grain than does lower protein wheat. In fact, wheat with 17 percent protein content provides more of each essential amino acid than does an equal quantity of 10 percent protein opaque-2 corn. We adjust the lysine values of our experimental wheat to a common protein level to permit valid lysine comparisons among wheats that differ in protein content.

Where in the wheat kernel do the high protein and high lysine effects reside?

We fractionated the grain of Nap Hal, Atlas 66, C.I.13449, and Centurk into starchy endosperm (white flour) and non-endosperm (bran) fractions for protein and lysine analyses. The results appear in Table 3. The high protein of Atlas 66 resides entirely in the starchy endosperm fraction of its grain, whereas in Nap Hal the high protein effect appears both in the starchy endosperm and in the non-endosperm fractions. All of the high protein effect of Atlas 66 and most of that of Nap Hal can be expected to be transmitted to white milled flour.

Table 3.--Protein and lysine content of whole grain, endosperm, and non-endosperm fractions of 4 wheat varieties grown at Yuma, Arizona in 1973

Variety	Protein content <sup>1</sup> (%)			Lysine content (% protein)		
	Whole grain	Endosperm fraction	Non-endosperm fraction	Whole grain	Endosperm fraction	Non-endosperm fraction
Nap Hal	19.6	18.9	24.5	3.1	2.5	4.6
Atlas 66	19.4	19.3	19.8	2.8	2.5	4.4
C.I.13449	15.5	14.5	19.6	3.1	2.8	4.4
Centurk	15.4	15.0	19.6	3.0	2.5	4.5
L.S.D. .05	1.0	0.9	1.0	ns	ns	ns

<sup>1</sup>Dry weight basis.

The higher-than-normal lysine of Nap Hal appears to reside entirely in the non-endosperm fraction of its seed. In contrast, the data suggest that the high lysine of C.I.13449 results primarily from elevated lysine in the starchy endosperm.

#### Does higher protein in wheat result in higher nutritive value?

Our data from small animal and human feeding trials indicate that it does. The results of a bioassay in which whole ground grain of 4 varieties differing in protein content constituted 56 percent of mouse diets appear in Table 4. The wheats with the highest protein content produced more favorable (smaller) FERs than the lower protein wheats.

Table 4.--FER values for whole ground grain of 4 wheat varieties differing in protein and lysine content evaluated by mouse bioassay

Variety	Protein	Lysine per	FER (56% grain in diet)	
	content (%)	unit protein (%)	$\bar{x}$	s.d.
Nap Hal	18.7	3.1	9.9	1.8
Atlas 66	17.5	2.9	9.8	1.8
Centurk	14.6	2.9	11.9	1.3
Bezostaya 1	13.2	2.8	14.3	4.2
Commercial sample	13.3	3.0	14.4	1.9
L.S.D. .05			3.7	

Correlations (n = 20)

Protein vs. FER = -.66\*\*

Lysine/protein vs. FER = -.21 (ns)

White milled flour from the same varieties also was bioassayed. Again, the mouse diets containing 56 percent flour from the varieties with the highest protein content provided better FERs than that from lower protein varieties (Table 5).

#### Is higher protein in wheat compatible with high productivity?

This year the Nebraska Agricultural Experiment Station and the Agricultural Research Service together with the Kansas, Texas, and South Dakota Experiment Stations jointly released the first variety from the ARS-Nebraska high protein breeding program. The performance of Lancota in Nebraska yield trials is shown in Table 6.

Table 5.--FER values for white milled flour from 4 wheat varieties differing in protein content evaluated by mouse bioassay

Variety	Flour		FER (56% flour in diet)	
	Protein (%)	Lysine per unit protein (%)	x	s.d.
Nap Hal	16.5	2.2	10.8	1.8
Atlas 66	17.2	2.1	12.6	1.6
Centurk	12.4	2.2	17.7	3.1
Bezostaya 1	11.1	2.0	25.3	10.6
Commercial sample	12.2	2.2	31.6	20.5
L.S.D. .05			15.8	

Correlations (n = 20)

Protein vs. FER = -.54\*\*

Lysine/protein vs. FER = -.09 (ns)

Table 6.--Performance of C.I.17389 in Nebraska statewide trials in 1973 and 1974

Variety	Grain yield (q/ha)			Protein content <sup>1</sup> (%)		
	1973	1974	2-yr. av.	1973	1974	2-yr. av.
Lancota	28.6	32.6	30.6	15.5	15.0	15.3
Centurk	29.3	32.6	31.0	13.9	13.7	13.8
Scout 66	29.3	31.3	30.3	13.9	13.8	13.9

<sup>1</sup>Dry weight basis.

Note that during 2 years of testing, Lancota was equally as productive as Centurk and Scout 66, our most popular and widely grown varieties in Nebraska. Its protein content averaged approximately 1.5 percentage point higher than Centurk and Scout 66. Additionally, Lancota produces grain with excellent milling and baking quality, and possesses effective field resistance to leaf and stem rust. Lancota exhibited its protein superiority over other comparably productive varieties in worldwide IWWPN trials in 1972 and 1973 (Table 7). Its protein advantage ranged from 1.1 to 2.3 percentage points.

Table 7.--Performance of C.I.17389 in the IWWPN in 1972 and 1973 (25 test sites)

Variety	Average yield	Average protein content
	(q/ha)	(%)
Lancota	40.7	15.5
Zenith	39.7	14.4
Centurk	43.9	14.0
TAM 102	39.9	13.5
Maris Nimrod	43.4	13.2

Correlation (yield vs. protein) r = -.13\*\*

Can the level of protein and its amino acid composition be further improved by breeding?

We crossed the two high protein varieties Nap Hal and Atlas 66 and obtained evidence of transgressive segregation for very high grain protein content among F<sub>2</sub> progeny rows grown at Yuma, Arizona. This suggested that the parent varieties contributed different genes for high protein which functioned

additively in the progeny to produce protein levels higher than in either parent. This has been substantiated in 3 years of evaluation of selections from the cross. Performance data from 1975 are summarized in Table 8.

Table 8.--Grain yield, protein, and lysine content of selected varieties and F<sub>7</sub> lines from Nap Hal/Atlas 66 grown at Yuma, Arizona in 1975

Population	No.	Protein (%)	Adj. lysine (% of protein)	Grain yield (bu/a)
Nap Hal/Atlas 66	13	16.8 (16.7-19.7)	3.3 (3.2-3.4)	56 (40-89)
Nap Hal	3	13.0 (12.7-13.4)	3.3 (3.2-3.4)	43 (25-52)
Atlas 66	3	14.4 (14.0-14.7)	3.1 (3.0-3.1)	94 (79-108)
Centurk	3	10.5 (10.0-10.8)	2.9 (2.7-3.0)	86 (60-102)
Lancota	3	13.9 (12.9-15.3)	3.0 (3.0-3.1)	117 (65-144)

Thirteen F<sub>7</sub> Nap Hal/Atlas 66 lines that ranged in yield from 40 to 89 bushels per acre averaged 16.8 percent protein compared to 10.5 percent for Centurk and 13.0 and 14.4 percent respectively for Nap Hal and Atlas 66, the parent varieties. Additionally, the Nap Hal level of lysine was recovered in the F<sub>7</sub> lines. Although the lines were substantially lower yielding than Atlas 66 or Centurk on the average, individual lines equal to these varieties in yield were found in which the protein content substantially exceeded either variety.

Similar evidence of transgressive segregation for high lysine was obtained from the cross Nap Hal/C.I.13449. Yield, protein, and lysine data for F<sub>6</sub> lines grown at Yuma, Arizona in 1975 are summarized in Table 9.

Table 9.--Grain yield, protein, and lysine content of selected varieties and F<sub>6</sub> lines from Nap Hal/C.I.13449 grown at Yuma, Arizona in 1975

Population	No.	Protein (%)	Adj. lysine (% of protein)	Grain yield (bu/a)
Nap Hal/C.I.13449	18	12.7 (12.0-14.8)	3.5 (3.4-3.6)	86 (37-125)
Nap Hal	4	13.0 (12.7-13.6)	3.3 (3.2-3.4)	38 (24-48)
C.I.13449	4	10.5 (10.2-11.3)	3.2 (3.1-3.3)	92 (76-118)
Centurk	4	10.1 (9.8-10.4)	2.9 (2.8-3.1)	68 (49-82)
Lancota	5	13.1 (12.7-14.2)	3.1 (2.9-3.3)	126 (121-135)

Eighteen lines that averaged 12.7 percent protein and with lysine values ranging from 3.4 to 3.6 percent averaged 86 bushels per acre. This compares with only 10.1 percent average protein, 2.9 percent average lysine and only 68 bushels per acre yield for Centurk. The lines were comparable to their Nap Hal parent in protein content and exceeded both Nap Hal and C.I.13449 in lysine content. They were comparable to C.I.13449 in grain yield.

I conclude from these data that there is excellent opportunity to significantly increase both the protein and lysine content of wheat over the level exhibited by Lancota. Further, our data suggest that such increases can be achieved in highly productive varieties with acceptable processing quality.

### Summary

Genetic variation for the protein content of wheat grain of approximately 5 percentage points has been identified among wheats in the USDA World Collection. Modest but useable genetic variation for lysine of approximately 0.5 percentage point has been identified.

Genetic factors and the production environment affect the protein content and amino acid composition of wheat grain. Fixed levels of protein in wheat cannot be achieved by breeding, but genetic potential for high protein is a heritable trait. Protein content of wheat is strongly affected by soil nitrogen availability. At most soil fertility levels, genes from Atlas 66 promote protein content approximately 2 percentage points higher than the protein content of comparably yielding ordinary varieties.

Increases in protein content of wheat grain up to 15 percent protein are associated with decreased lysine content of the protein. Further increases in protein content above 15 percent do not affect lysine level. Higher protein in wheat increases the amount of lysine per unit weight of grain.

Results from weanling mice bioassays indicate that high protein wheat is more nutritious than lower protein wheat.

Genes for high protein from Atlas 66 were successfully transferred to a productive, disease-resistant new hard red winter wheat with excellent milling and baking quality. The Lancota variety released jointly by ARS, Nebraska, Kansas, South Dakota, and Texas in 1975, produces grain with 1 to 2 percentage points higher protein content than ordinary varieties under Nebraska conditions. Lancota has been equal in productivity to the popular Centurk and Scout 66 varieties.

Genes for high protein different from those in Atlas 66 have been identified and, when combined with the Atlas 66 genes, produce protein levels higher than that of Atlas 66. Most of the high protein effect resides in the starchy endosperm fraction of the wheat kernel and is retained in white milled flour. Transgressive segregation for high lysine was detected in a cross of two wheats, Nap Hal and C.I.13449, both of which exhibit modestly elevated lysine. Lines have been selected from this cross that combine high lysine with high grain protein.

## PROTEIN ELECTROPHORESIS AIDS IN WHEAT BREEDING

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Breeding improved wheat is a formidable task: Any new varieties should improve baking or milling performance, give higher yield or protein contents, increase disease or pest resistance, or provide other specific agronomic characteristics. Improved wheats may arise through natural mutations and be detected by screening existing populations. Other mutations can be induced, either by chemical means or by ionizing radiation. Carefully controlled additions, subtractions, and blendings of different genetic materials may also lead to better varieties. To detect such improved wheats among many, usually small, samples, the breeder needs rapid and specific methods of analysis.

One such method, protein analysis, has become increasingly useful to detect variability resulting from chemical changes in chromosomes. The chromosome's basic units, the genes, store the information for producing proteins, which influence wheat's quality and properties. Consequently, differences in proteins, which indicate genetic variability, may be used as markers of known characteristics. Rapid, specific, and sensitive methods of protein analysis should be most useful to a breeder. Today I shall discuss such a method developed at the Northern Laboratory for rapidly analyzing proteins from single wheat kernels (1), show how this method has suggested that good dough strength and baking properties may be related to specific proteins, and give other examples of its use.

Wheat Protein Separation. Because wheat proteins are both numerous and complex, usually for study they are divided into simpler classes. Originally, Osborne described four classes of wheat proteins (Figure 1), a classification changed little to this day. Wheat albumins, which are water soluble, and globulins, which are soluble in dilute salt solutions, make up the major proteins of wheat germ. Many of these proteins are enzymes, involved in biosynthetic and metabolic activities of the plant. Wheat endosperm contains primarily gliadin, which is soluble in 70% ethanol, and glutenin, which is soluble only in dilute solutions of acid or alkali; together, these proteins constitute gluten. Some other proteins that do not dissolve readily with any of these solvents remain in the starchy residue; although these proteins are mainly glutenin (2), they are probably less soluble because they have higher molecular weights (MW).

Studies on Glutenin. Glutenin is the wheat flour protein most important in giving strength and elasticity to dough (3), and much of our wheat protein research at the Northern Laboratory over recent years has dealt with this protein class. We first began to understand the nature of glutenin better through the technique of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Figure 2). In this method, disulfide bonds of proteins are cleaved with 2-mercaptoethanol (ME), and the reduced subunits are complexed with the anionic detergent SDS. All subunits, or fragments of the intact molecules, then exist as rodlike particles of constant diameter, with lengths proportional to their MW. When these protein-detergent complexes are

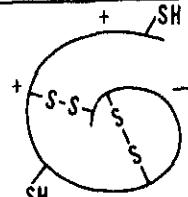
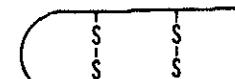
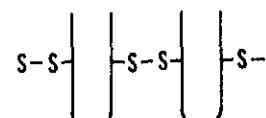
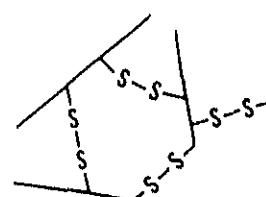
Class	Solubility	Features
Albumins and Globulins	Salt Solutions	
Gliadin	70% Alcohol Solution	
Glutenin	1% Acetic Acid	
Residue	Reducing Agents or Alkali	

Figure 1. Classes of proteins in wheat flour.

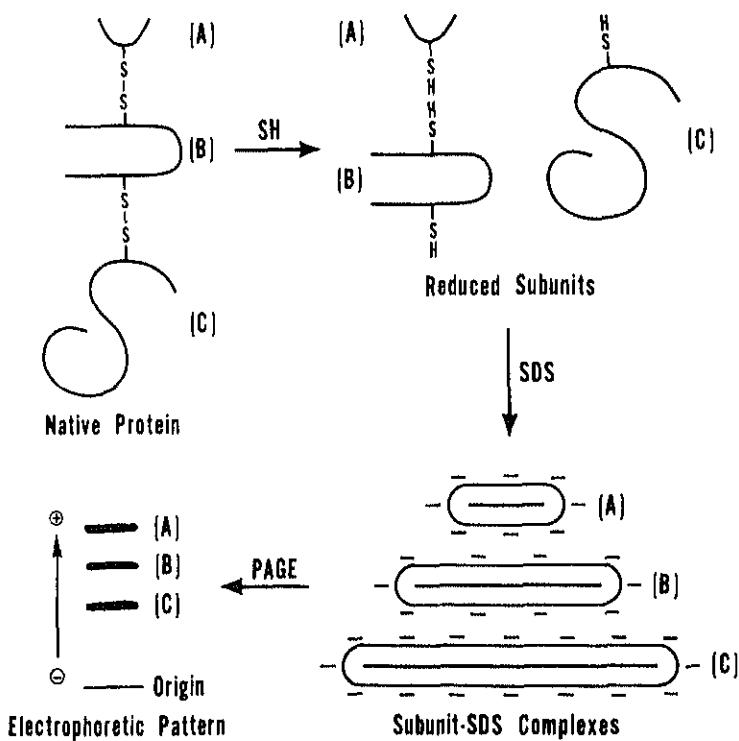


Figure 2. Principles of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of proteins.

placed in a polyacrylamide gel and an electric current is applied, they migrate at a rate determined by the ease with which they penetrate the gel; this movement is related to their MW. Large subunits migrate slowly, small ones quickly. In this manner, the number and approximate MW of all subunits in a complex mixture can be estimated (Figure 3). By the SDS-PAGE method,

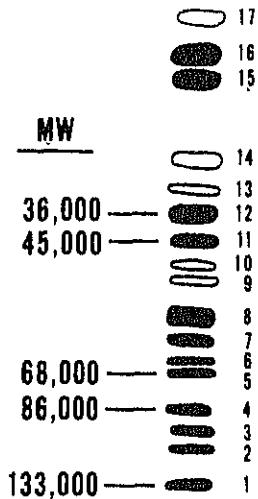


Figure 3. SDS-PAGE of glutenin from the common wheat variety Chinese Spring. Numbering system and representative molecular weights (MW) of subunits are indicated.

glutenin is seen to consist of many polypeptides of differing MW, which in the native molecule were joined together by disulfide bonds (4). Some of glutenin's subunits are ethanol-soluble and closely resemble some gliadin proteins (5), but most, including those of high MW, are unique to glutenin.

Single-Kernel Method. Since glutenin is essential in giving wheat its properties, we examined its subunit composition in many different varieties and lines. Limited amounts of many wheat stocks were available, however (as would be typical early in a breeding program), so we developed a new method (1) for examining glutenin from single kernels of wheat (Figure 4).

After the germ is cut off with a razor blade, the remainder of the kernel is ground with a mortar and pestle. The germ may be saved and grown, if desired. Glutenin is then completely freed of all other proteins by sequential extraction, as diagrammed on the right side of Figure 4. After each extraction, glutenin remains in the starchy residue and is only solubilized upon incubation with a solution containing SDS and ME. After centrifugation, the supernatant can be analyzed by SDS electrophoresis to reveal number and MW of proteins and to compare samples. This method yields the same information

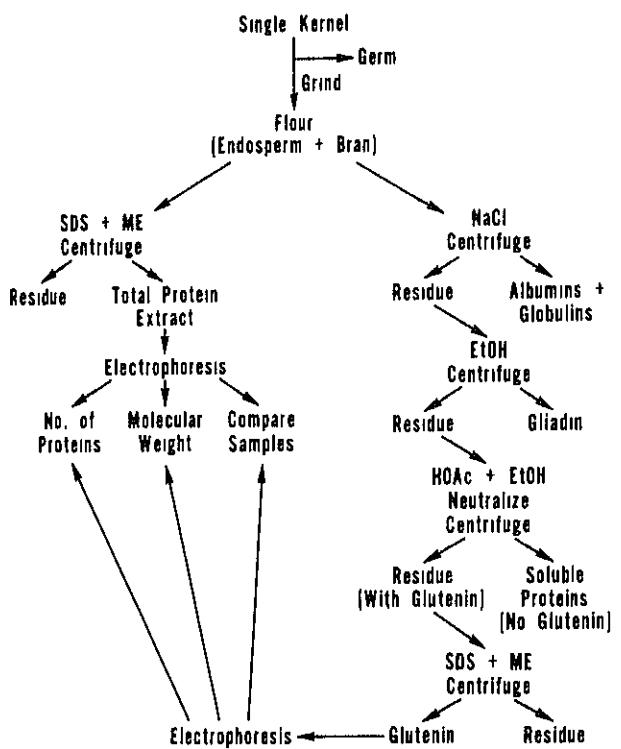


Figure 4. Single-kernel extraction of wheat proteins and analysis by SDS-PAGE after cleavage with 2-mercaptoethanol (ME).

obtained from bulk samples. Alternatively, the entire flour may be incubated with SDS and ME, and all proteins may be extracted and examined. With this new method, several hundred samples can be analyzed within a few days.

Chromosomes Coding Glutenin Subunits. We first used the method of single kernel analysis to determine which wheat chromosomes contain genes that code glutenin subunits, particularly its unique ones of high MW. Figure 5

	Chromosome Number						
	1	2	3	4	5	6	7
Genome	A						
	B						
	D						

Figure 5. Chromosomes of hexaploid wheat.

diagrams the chromosomes of hexaploid bread wheat. Three closely related sets of chromosomes, called A, B, and D genomes, are present. Each genome contains 7 pairs of chromosomes, so that wheat has a total of 42 chromosomes. Since each protein chain is coded by one gene on an individual chromosome, a wheat

line in which a pair of identical chromosomes is removed will lack all proteins coded by genes on that chromosome. A series of such wheat lines, which are genetic variants of the variety Chinese Spring deficient in chromosome pairs or in parts of chromosomes, has been developed by Dr. E. R. Sears (USDA-ARS, University of Missouri, Columbia), and we used it for our study. For example, lines were available that lacked both 1B chromosomes or both 1D chromosomes. By comparing the subunit composition of all such lines to that of the parent variety, we determined which chromosomes coded which polypeptides (Figure 6).

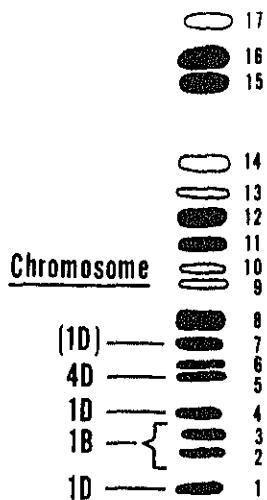


Figure 6. Chromosomes controlling synthesis of glutenin subunits in common wheat. Numbering system and chromosomes coding high MW subunits are indicated.

Chromosomes 1D, 1B, and 4D were found to code glutenin's five highest MW subunits. In any line in which one of these chromosome pairs was absent, the protein subunits that it codes were also missing. These glutenin subunits, then, can serve as markers of the presence or absence of wheat chromosomes 1B, 1D, and 4D and, therefore, as a basis for selection. For example, if these chromosomes are known to be related to certain desirable or undesirable characteristics, lines with such properties may be detected through electrophoretic analysis of glutenin at the seed level, rather than on a bulk sample or a mature plant. Also, since these high MW subunits occur only in glutenin and since many of wheat's good baking quality characteristics are associated with chromosomes 1D and 1B, we suggest that these high MW subunits may be essential, although certainly not sufficient (since they are also present in poor varieties), for good wheat quality through participation in forming a strong gluten network.

Common (Bread) Wheat Varieties. We have now used the single-kernel technique to examine many varieties of bread and durum wheats and some related species. I shall describe some of our more interesting findings, as well as give examples of how protein electrophoresis may be useful to wheat breeders.

First, however, let us briefly examine the origin of wheat and some related cereals (Figure 7). An unknown 14-chromosome precursor gave rise to

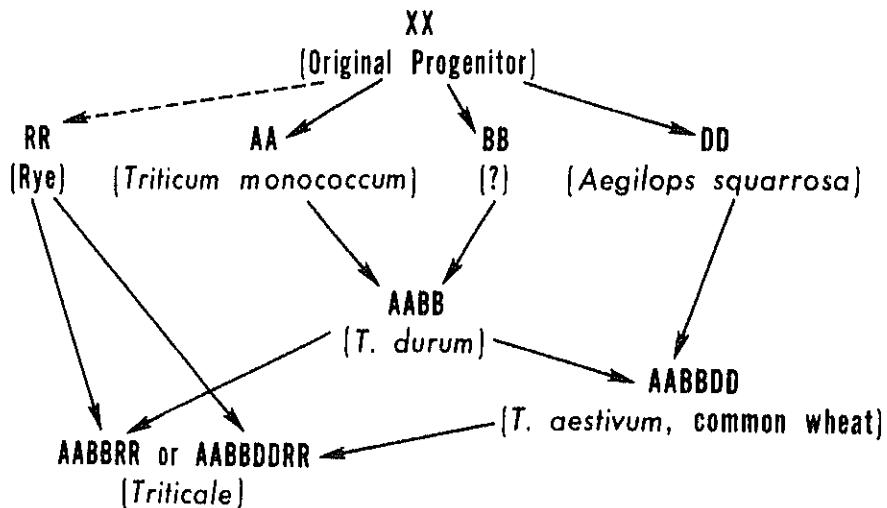


Figure 7. Probable origin of wheat.

several closely related offspring, represented here by the different genomes. These include the R genome of rye, the A genome of Triticum monococcum, an unknown species with the B genome, and the D genome of Aegilops squarrosa. At some time, the A and B genomes hybridized to an AABB tetraploid wheat similar to Triticum durum; later, the D genome was added to produce hexaploid bread wheat, Triticum aestivum, which differs from durum wheat only by the presence of 14 additional D-genome chromosomes. Since rye is also closely related, it can hybridize with either bread or durum wheat to form Triticale.

After examining glutenin in Chinese Spring, we wondered how much variability in subunit patterns occurred among other wheats. Consequently, we examined many common wheat varieties of different quality, characteristics, and geographical origins. A gel comparing many varieties, typical of results obtained with the single-kernel technique, is reproduced as Figure 8. For simplicity, some results showing only glutenin's high MW subunits are diagrammed in Figure 9.

In one group of 80 varieties, about 75 had subunits essentially identical to those of Chinese Spring. This suggests that glutenin subunit coding is indeed similar in most hexaploid wheat varieties. We also found four varieties in which an additional high MW subunit was present; an example is Hokuei (Figure 9b). Because these varieties may differ in quality or agronomic characteristics from most other lines, screening for such subunit patterns may be of value.

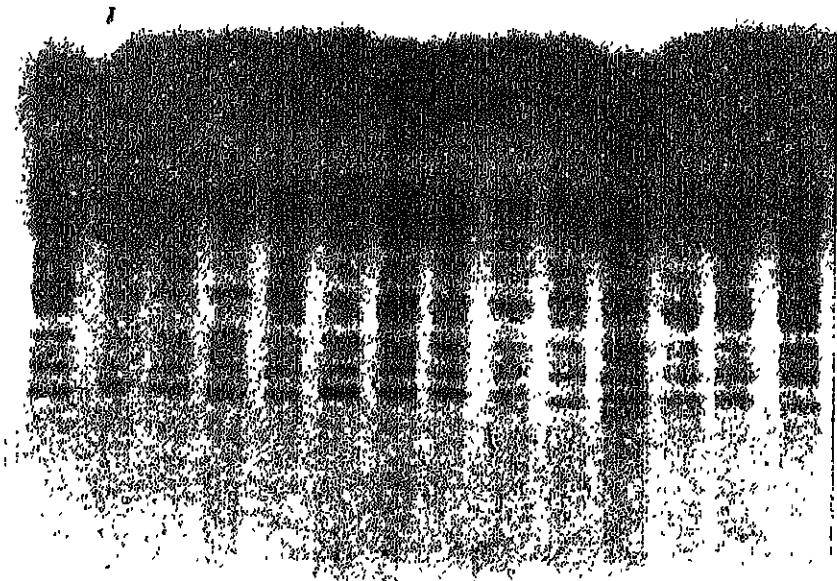


Figure 8. Typical SDS-PAGE electrophorogram comparing hexaploid wheat varieties.

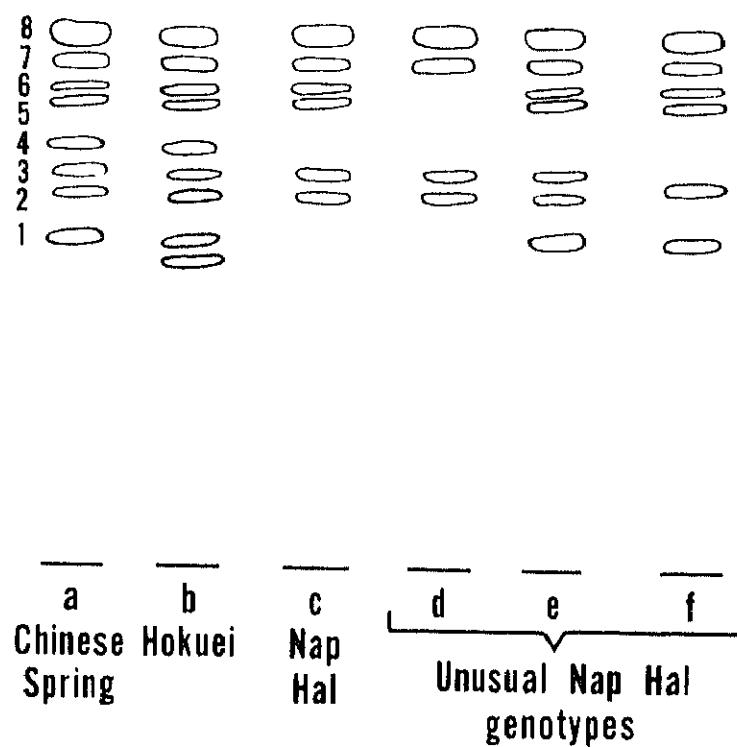


Figure 9. High MW glutenin subunits in the hexaploid wheat varieties:  
 (a) Chinese Spring, (b) Hokuei, (c) Nap Hal, and (d-f) unusual Nap Hal genotypes.

One variety, Nap Hal (Figure 9c), was unusual because subunits 1 and 4 are absent. Preliminary cytological analysis revealed a normal chromosome number, although it may be possible that a fragment of chromosome 1D, which codes these subunits, is absent. Interestingly, Nap Hal has high-protein and high-lysine contents (6) but bad baking properties, possibly related to the absence of these two subunits. Nap Hal may, however, prove useful in further genetic or breeding studies.

A bulk sample of Nap Hal contained a few larger, deeper creased, more wrinkled kernels, which also had unusual glutenins (Figure 9d-f). The germ ends of these kernels are being grown and will be examined further for unusual properties. Our study shows that the single kernel technique is useful for examining bulk samples and that lines with unusual genotypes can be selected by unusual kernel appearance.

Durum Wheat Varieties. Typical glutenin subunit patterns from a group of 55 durum wheat varieties are shown in Figure 10. Since durum wheats lack all

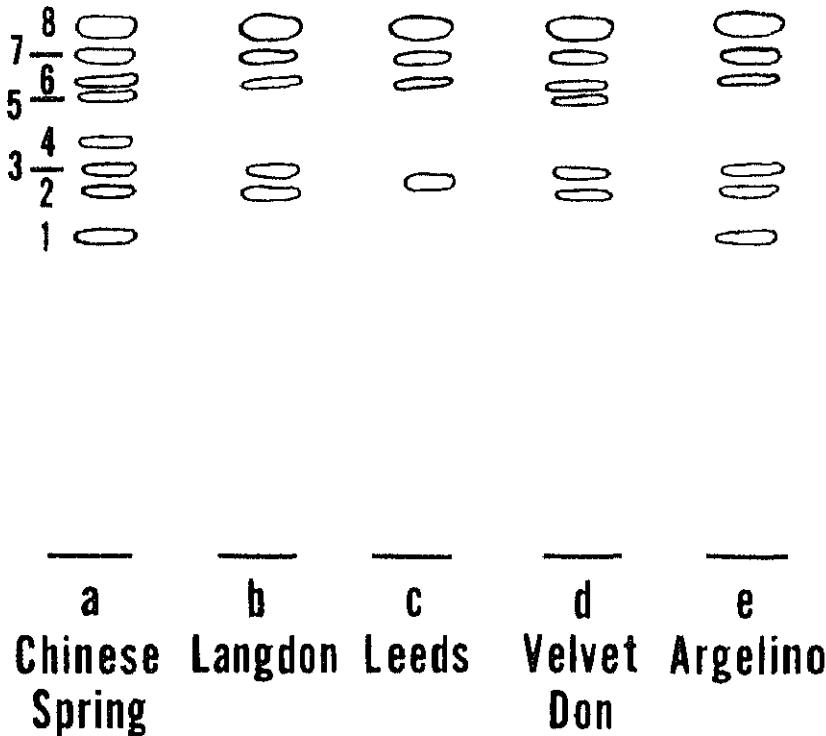


Figure 10. High MW glutenin subunits in durum wheat varieties:  
 (a) Chinese Spring (standard hexaploid variety),  
 (b) Langdon, (c) Leeds, (d) Velvet Don, and (e) Argelino.

D genome chromosomes, we expected subunits 1, 4, and 5, coded by 1D and 4D, to be absent. Only 18 of the 55 varieties, such as Langdon (Figure 10b), had this type of composition, however. In 18 other varieties, such as Leeds (Figure 10c), subunits 2 and 3 differed greatly or only one was present. Six varieties, including Velvet Don (Figure 10d), contained a subunit similar to that coded by chromosome 4D. Finally, 12 varieties, like Argelino (Figure 10e), had glutenin subunits similar to bread wheat's highest MW subunit; if

this subunit strongly influences bread wheat's properties, these durum varieties could have superior properties. Cytological analysis of these varieties showed that all were indeed durum wheats; their characteristics and spaghetti-making properties are being further evaluated by Dr. Leonard Joppa (USDA-ARS, State University Station, Fargo, ND). We believe that varietal selection through glutenin analysis promises to be a valuable tool to the durum wheat breeder.

Glutenin subunits may also serve as markers for the presence or absence of specific chromosomes in durum breeding programs. A durum variety, Camara (Figure 11c), is now available (7) in which chromosome 1B is replaced by 1D.

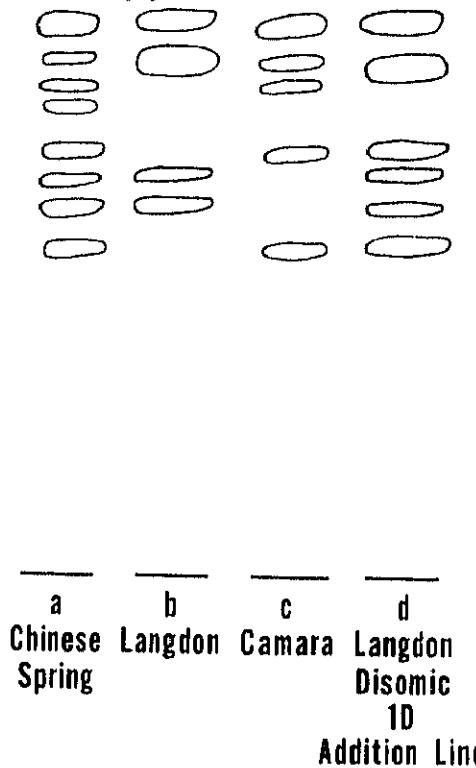


Figure 11. Glutenin analysis in durum wheat breeding: (a) Chinese Spring (standard hexaploid variety), (b) Langdon, (c) Camara, and (d) Langdon disomic 1D addition line.

A similar substitution line has also been developed by Joppa\*. In both lines, the subunits coded by 1B are absent, but those coded by 1D are present. Joppa has also developed a 30 chromosome addition line of the durum variety Langdon (8). Analysis of its glutenin subunit composition (Figure 11d) by SDS electrophoresis indicated that two high MW subunits coded by 1D were present and confirmed that this sample was a disomic 1D addition line. In these examples, a simple protein electrophoretic technique confirmed, and even substituted for, more difficult cytological evaluation techniques.

Semolina milled from the 1D addition line has strong mixing properties compared to its durum wheat parent (Figure 12); this characteristic could

\*L. R. Joppa, personal communication.

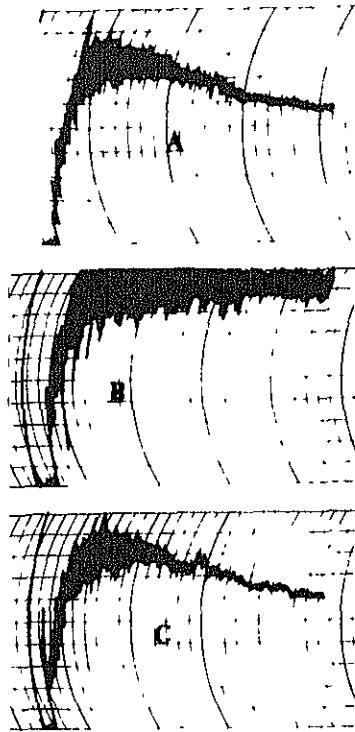


Figure 12. Durum wheat semolina micro-mixograms: (a) Langdon durum, (b) 1D-disomic addition line to Langdon durum, and (c) sister line of same parentage as (b), but without 1D chromosomes.

improve cooking quality and firmness in spaghetti. Also, the two high MW glutenin polypeptides could improve bread-baking qualities of durum flour. The 1D addition line may also have higher protein than its durum parent, further increasing its potential. Careful manipulation of chromosomes may allow the geneticist and breeder to improve wheat's quality and nutritive content, and protein analysis represents an effective tool in reaching this goal.

Value in Genetic Studies. Single-kernel analysis of glutenin should be valuable to the wheat geneticist, as well as the breeder (Figure 13). First, let us look at the derived AABB tetraploid wheats (9), which result when all D-genome chromosomes are removed from bread wheats. Comparing these materials is valuable in determining how D-genome chromosomes give bread wheat its useful properties. When we compared Prelude wheat (Figure 13b) with Tetraprelude (Figure 13c), its derived AABB tetraploid, we found that glutenin subunit 1 was still present. The presence of this subunit suggested that part of the 1D chromosome was in the tetraploid, and seemed to confirm other evidence that a part of the 1D chromosome has actually been incorporated into an A or B genome chromosome of Tetraprelude (10). We also examined glutenins from some wheat ancestors. For example, Aegilops squarrosa (Figure 13d), wheat's D genome donor, has glutenin subunits basically the same as those coded by D genome chromosomes in hexaploid wheat. Triticum monococcum (Figure 13e), however, which is the A genome donor in hexaploid wheat, has a major high MW subunit not present in hexaploid wheat. Either the gene coding this subunit

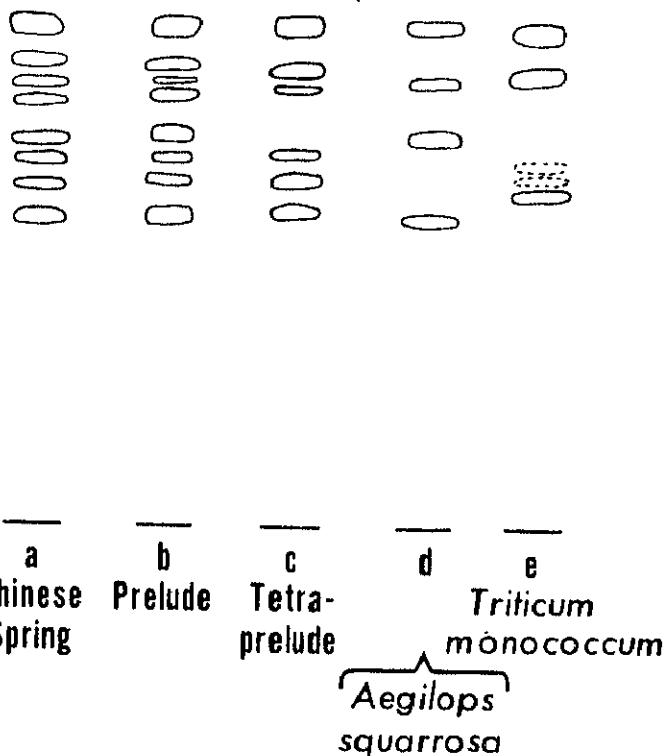


Figure 13. Genetic applications of glutenin analysis: (a) Chinese Spring, (b) Prelude, (c) Tetraprelude, (d) Aegilops squarrosa, and (e) Triticum monococcum.

lost during evolution, the species has changed, or this variety differs that actually incorporated into hexaploid wheat. At any rate, glutenin analysis through electrophoresis aids the study of wheat and its evolution.

Single-kernel analysis of glutenin is also useful in analyzing hybrids resulting from crossing related lines (Figure 14). For example, the wheat variety Chinese Spring was hybridized with Imperial rye. Glutenin of the hybrid (Figure 14b), which contains all chromosomes from both varieties, contains all subunits of both parents (Figure 14, a and c). This SDS-PAGE method can be used to differentiate hybrid wheats or Triticale from their parent(s).

Other Uses of Protein Electrophoresis. Although I have only discussed glutenin proteins studied through SDS electrophoresis, albumin, globulin, and other protein classes may also reveal desired changes and relationships to the breeder; and other electrophoretic techniques may also be superior to SDS electrophoresis for these protein classes. Generally, any protein capable of differentiating varieties or of being related to a specific characteristic can be easily detected by some kind of fairly simple electrophoretic procedure. Of course, protein electrophoresis will not always be the best practical method. Rather, it is a powerful tool for detecting and analyzing variability and specific characteristics, both in early and late generations, and needs to be used along with all other tools the breeder possesses.

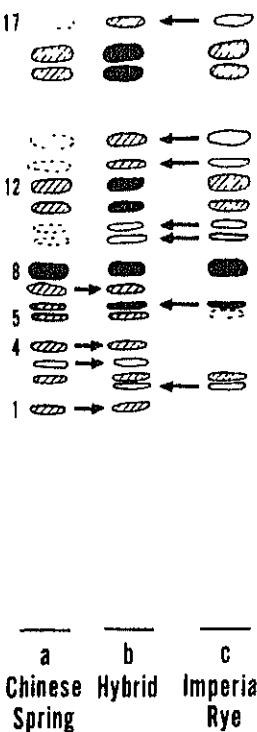


Figure 14. Glutenin analysis in a wheat-rye hybrid: (a) Chinese Spring wheat, (b) Chinese Spring X Imperial hybrid, and (c) Imperial rye.

Many others have, of course, used protein electrophoresis in wheat breeding and genetics; the following are examples of its general value.

Origin of Wheat. Protein electrophoresis has revealed much about wheat's origin and ancestors, since specific proteins are uniquely associated with specific genera or species. The identities and geographical origins of wheat A and D genome donors were studied through polyacrylamide gel electrophoresis of albumin, globulin, and gliadin proteins extracted with 70% ethanol (11). Enzymes have also been analyzed to reveal further the phylogeny of wheat (12, 13).

Marker Proteins. Electrophoretic analysis has also located genes for individual gliadins (14, 15), non-gliadin proteins (16), and enzymes (17, 18) on specific chromosomes. These proteins, therefore, serve as markers of individual chromosomes, which in turn can be related to specific characteristics. For example, in our studies high MW glutenin subunits may be associated with wheat's strength and baking quality characteristics.

Wheat Quality. Wheat quality has many different meanings for different uses; nevertheless, many attempts have been made to relate quality, through electrophoresis, to protein composition. Gliadin electrophoretic patterns vary considerably among varieties, but it is not certain whether they are (19, 20) or are not (21-23) related to bread baking quality. Bread wheat quality is not related to glutenin subunits either when examined by SDS electrophoresis

(1, 24) or by starch gel electrophoresis (25). Durum wheat quality, however, may be related to glutenin subunits (26).

Varietal Identification. Wheat breeders can often identify varieties by starch gel electrophoresis of gliadin proteins (27). Electrophoresis may also reveal varietal relationships among wheats, such as among many Australian varieties (28) and for wheats containing alpha-gliadin proteins (29).

Alien Chromosomes. In some breeding programs, "alien" chromosomes from different, though related, lines may replace wheat chromosomes to modify specific characters. For example, protein electrophoresis has been used to determine which chromosomes code the gliadinlike proteins of rye and of Aegilops umbellulata (30) and which wheat chromosomes these alien chromosomes may replace. In another alien species, protein electrophoresis demonstrated that gene transfer had occurred from Aegilops ventricosa to hexaploid wheat in a test cross (31).

Triticale and Hybrid Wheats. Electrophoresis has also revealed that triticale proteins are a sum of proteins from both the wheat and the rye parents (32, 33), as we observed with a wheat-rye hybrid. A practical application of this technique has been discovered: A breeder interested in marketing hybrids could use electrophoretic evidence to ascertain the percentage of hybrid seeds in a mixed population for the purpose of certification.

#### SUMMARY

Protein analysis by electrophoresis can be extremely useful to the wheat breeder. For example, glutenin subunits from single wheat kernels may be analyzed by SDS electrophoresis; these techniques are highly specific, reproducible, and rapid and are particularly applicable to early generation screening and selection. Other wheat protein classes may also be analyzed and compared by other electrophoretic techniques. The breeder can use protein electrophoresis to detect variability through screening, to look for mutation or variability within a variety, and to relate specific marker proteins to specific characteristics, properties, or possibly to quality. The wheat geneticist also can use these techniques to study evolutionary relationships among related species or between varieties. Protein electrophoresis should become a valuable addition to all other methods of selection and analysis used by wheat breeders and geneticists.

#### Literature Cited

1. Bietz, J. A., Shepherd, K. W., and Wall, J. S. Single-kernel analysis of glutenin: Use in wheat genetics and breeding. *Cereal Chem.* 52:513, 1975.
2. Bietz, J. A. and Wall, J. S. The effect of various extractants on the subunit composition and associations of wheat glutenin. *Cereal Chem.* 52:145, 1975.
3. Bietz, J. A., Huebner, F. R., and Wall, J. S. Glutenin--the strength protein of wheat flour. *Bakers' Dig.* 47(1):26, 1973.

4. Bietz, J. A. and Wall, J. S. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Cereal Chem.* 49:416, 1972.
5. Bietz, J. A. and Wall, J. S. Isolation and characterization of gliadin-like subunits from glutenin. *Cereal Chem.* 50:537, 1973.
6. Johnson, V. A., Mattern, P. J., and Schmidt, J. W. Genetic studies of wheat proteins. *Symposium: Seed Proteins*, ed. by G. E. Inglett; p. 126. Avi Publishing Co., Westport, Conn., 1972.
7. Mello-Sampayo, T. "Camara" a tetraploid wheat carrying a 1D disomic substitution for chromosome 1B. *Wheat Info. Serv.* 37:5, 1973.
8. Joppa, L. R., Bietz, J. A., and McDonald, C. Development and characteristics of a disomic-1D addition line of durum wheat. *Can. J. Genet. Cytol.*, in press.
9. Kerber, E. R. Wheat: Reconstitution of the tetraploid component (AABB) of hexaploids. *Science* 143:253, 1964.
10. Kaltsikes, P. J., Evans, L. E., and Bushuk, W. Durum-type wheat with high bread-making quality. *Science* 159:211, 1968.
11. Johnson, B. L. Seed protein profiles and the origin of the hexaploid wheats. *Am. J. Bot.* 59:952, 1972.
12. Bozzini, A., Cubadda, T., and Quattrucci, F. Esterases in *Triticum* and some related species. *Proc. 4th Int. Wheat Genet. Symp. (Univ. Mo., Columbia)*:783, 1973.
13. Wolf, G. and Lerch, B. Genome analysis in the *Triticinae* using isoenzymes of phosphodiesterase. *Proc. 4th Int. Wheat Genet. Symp. (Univ. Mo., Columbia)*:885, 1973.
14. Shepherd, K. W. Chromosomal control of endosperm proteins in wheat and rye. *Proc. 3rd Int. Wheat Genet. Symp. (Aust. Acad. Sci., Canberra)*:86, 1968.
15. Wrigley, C. W. and Shepherd, K. W. Electrofocusing of grain proteins from wheat genotypes. *Ann. N.Y. Acad. Sci.* 209:154, 1973.
16. Aragoncillo, C., Rodriguez Loperena, M. A., Carbonero, P., and Garcia-Olmedo, F. Chromosomal control of non-gliadin proteins from the 70% ethanol extract of wheat endosperm. *Theo. Appl. Genet.* 45:322, 1975.
17. Hart, G. E. Homoeologous gene evolution in hexaploid wheat. *Proc. 4th Int. Wheat Genet. Symp. (Univ. Mo., Columbia)*:805, 1973.
18. May, C. E., Vickery, R. S., and Driscoll, C. J. Gene control in hexaploid wheat. *Proc. 4th Int. Wheat Genet. Symp. (Univ. Mo., Columbia)*:843, 1973.

19. Doeke, G. J. Comparison of wheat varieties by starch-gel electrophoresis of their grain proteins. *J. Sci. Food Agric.* 19:169, 1968.
20. Sozinov, A. A., Popereya, F. A., and Stakanova, A. I. Use of electrophoresis of gliadin for selection of wheat by quality. *Vestn. Sel'skokhoz. Nauki* (Moscow) 7:99, 1974; *Chem. Abstr.* 81:166298, 1974.
21. Huebner, F. R. and Rothfus, J. A. Gliadin proteins from different varieties of wheats. *Cereal Chem.* 45:242, 1968.
22. Orth, R. A. and Bushuk, W. A comparative study of the proteins of wheats of diverse baking qualities. *Cereal Chem.* 49:268, 1972.
23. Tanaka, K. and Bushuk, W. Effect of protein content and wheat variety on solubility and electrophoretic properties of flour proteins. *Cereal Chem.* 49:247, 1972.
24. Orth, R. A. and Bushuk, W. Studies of glutenin. II. Relation of variety, location of growth, and baking quality to molecular weight distribution of subunits. *Cereal Chem.* 50:191, 1973.
25. Huebner, F. R. Comparative studies on glutenins from different classes of wheat. *J. Agric. Food Chem.* 18:256, 1970.
26. Wasik, R. J. and Bushuk, W. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reduced glutenin of durum wheats of different spaghetti-making quality. *Cereal Chem.* 52:328, 1975.
27. Wrigley, C. W. and Shepherd, K. W. Identification of Australian wheat cultivars by laboratory procedures: Examination of pure samples of grain. *Aust. J. Exp. Agric. Anim. Husb.* 14:796, 1974.
28. Moss, H. J. and Wrigley, C. W. Interrelationships between the pedigrees of Australian wheats. *J. Aust. Inst. Agric. Sci.*, p. 207, September 1974.
29. Kasarda, D. D., Qualset, C. O., and Platt, S. G. Varietal relationships of the alpha-gliadin proteins in wheat. *Proc. 4th Int. Wheat Genet. Symp.* (Univ. Mo., Columbia):811, 1973.
30. Shepherd, K. W. Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. *Proc. 4th Int. Wheat Genet. Symp.* (Univ. Mo., Columbia):745, 1973.
31. Delibes, A. and Garcia-Olmedo, F. Biochemical evidence of gene transfer from the M<sup>V</sup> genome of Aegilops ventricosa to hexaploid wheat. *Proc. 4th Int. Wheat Genet. Symp.* (Univ. Mo., Columbia):161, 1973.
32. Chen, C. H. and Bushuk, W. Nature of proteins in Triticale and its parental species. III. A comparison of their electrophoretic patterns. *Can. J. Plant Sci.* 50:25, 1970.
33. Orth, R. A., Dronzek, B. L., and Bushuk, W. Studies of glutenin, VII. Inheritance of its physicochemical factors in triticale. *Cereal Chem.* 51:281, 1974.

CHANGES IN PRODUCTION AND UTILIZATION OF  
DURUM WHEAT AND DURUM PRODUCTS

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Durum wheat (*Triticum durum*), which was first introduced from Russia into the U.S.A. in 1856 as the variety Arnautka (1), has seen considerable changes in its variety (cultivar) development, production and utilization since that time. These changes were not accidental. They were the result of natural and human forces which, over time, have had an enormous impact on the durum situation. It is the purpose of this paper to briefly review some of these developments and indicate those events which have brought durum wheat production and utilization to the point it is at today. It should be emphasized that durum wheat is the raw material of choice for producing the highest quality pasta products. By pasta products is meant such foods as macaroni, spaghetti and noodles each of which can be marketed in a wide variety of shapes and sizes.

Durum Production in the U.S.A.

The acreage of durum wheat grown in the U.S. fluctuates annually, primarily for two reasons namely price and planting conditions. The planted acreage, production and yield of durum in the U.S. since 1960 are shown in Table 1. As can be seen from the data, the acreage, production, and yield levels have shown substantial swings over the past 15 years. For example the average yield of 19.8 bushels per acre in 1974 was the lowest since 1961. This was due to late planning because of rain followed by a mid-summer drought. In 1970 the low production level was the result of wheat allotments, lower prices for durum and a large stock carryover from the previous Spring.

The importance of the durum export market has manifested itself over the past 15 years. Prior to 1960 this market was insignificant. However with the formation of Great Plains Wheat, Inc. and Wheat Associates, U.S.A., the promotion of the five American wheat classes around the world has had a very significant impact on the level of durum exports (Table 2). This, coupled with increasing domestic consumption of macaroni products has led to increased demand for durum wheat (Table 3). Both the domestic and export markets have benefited by this trend which increased significantly the total production of durum wheat.

The per capita consumption figures for macaroni products, shown in Table 3, should not be taken as being truly related to durum usage. In order to cut costs, particularly over the past couple of years, many macaroni manufacturers are using semolina blended with wheat flour. How much and to what extent blending is being used no one really knows. The actual data are just not available. Even the mill grind figures are questionable. As

Table 1. U.S. durum acreage, production and yield, 1960-1975\*

Year	Acres Planted(10 <sup>6</sup> )	Production Bushels (10 <sup>6</sup> )	Yield (Bu/acre)
1960	1.64	34.1	20.8
1961	1.54	19.0	12.3
1962	2.42	71.8	29.7
1963	1.99	50.4	25.7
1964	2.38	66.7	28.0
1965	2.23	69.9	30.8
1966	2.44	63.2	25.9
1967	2.75	66.4	24.1
1968	3.56	99.5	27.9
1969	3.33	106.3	31.9
1970	2.11	52.8	25.1
1971	2.86	91.8	32.1
1972	2.59	72.9	28.6
1973	2.95	78.5	29.5
1974	4.07	79.2	19.8
1975**	4.51	120.0	26.6

\*Data from the Wheat Situation, Economic Research Service, USDA.

\*\*Estimate (9-1-1975).

Table 2. U.S. durum production, consumption and carryover, 1960-1974 (Million bushels)\*

Year Beginning July	Production	Consumption			Carryover
		Domestic	Export		
1960	34.1	26	6		20
1961	19.0	20	16		5
1962	71.8	25	4		46
1963	50.4	28	29		41
1964	66.7	31	10		67
1965	69.9	50	34		55
1966	63.2	41	47		30
1967	66.4	41	31		24
1968	99.5	37	46		41
1969	106.3	35	34		80
1970	52.8	36	39		58
1971	91.8	37	44		69
1972	72.9	40	65		37
1973	78.5	47	42		28
1974	79.2	38	49		20

\*Data from the Wheat Situation, Economic Research Service, USDA.

was pointed out in the August edition of the Macaroni Journal by Melvin Sjerven (2) "Confusion prevails in reporting by mills, some listing total production for shipment to macaroni manufacturers, whether it be 100% durum or any other kind of a blend, others listing only the durum".

Table 3. U.S. macaroni consumption, 1965-1974\*

Year	Mill Grind	Domestic	Per Capita
	Durum	Consumption lbs ( $10^6$ )	Consumption lb
1966	29038	1376	7.02
1967	28538	1345	6.78
1968	28368	1410	6.95
1969	29762	1522	7.43
1970	32052	1599	7.72
1971	33236	1670	8.0
1972	33621	1760	8.33
1973	37622	1832	8.73
1974**	32775	1884	8.93

\*Source: Macaroni J. 55(4), 18 (1974).

\*\*Estimate.

Durum wheat is produced primarily in five states within the continental U.S. In decreasing order of production these are North Dakota, Montana, South Dakota, Minnesota and California. This past year (1974-1975) approximately one hundred thousand acres in the Yuma Valley in southern Arizona were devoted to durum production (3). It is further expected that durum production in Arizona will increase to three hundred thousand acres this coming year (1975-1976). Although in general the external appearance of the Arizona produced durum is quite acceptable, the quality as assigned by established criteria is inferior to that produced in North Dakota and certain other areas. The major deficiencies are a relatively lower wheat and semolina protein content and the poor color of the processed spaghetti.

#### Durum Production in North Dakota

The primary producer of quality durum wheat in the U.S. is North Dakota. This is due to the varieties seeded and a unique combination of environmental and agronomic growing conditions. In any one year since 1961 North Dakota has accounted for anywhere from 83 to almost 90% of the total U.S. durum production (Table 4). Do these varietal, environmental and agronomic conditions account solely for the large production levels in North Dakota? The answer of course is no! There are a combination of factors which contribute to North Dakota's preeminent position in this regard. Among these are: the cooperative role played by the Departments of Agronomy, Plant Pathology, Soils, Entomology and Cereal Chemistry and Technology within the College of Agriculture and the Agricultural Experiment Station at North Dakota State University; the availability of more than 8000 acres of land at eight locations within the state which affords for variety development to suit the state's climatic and agronomic needs in the most efficient way possible; the activities of the North Dakota State Wheat Commission which develops new market outlets, generates an awareness of pasta products and assists, with funds, the research efforts at NDSU; the pasta manufacturing industry which has promoted pasta utilization and has also cooperated and

Table 4. U.S. and North Dakota durum acreage and production, 1960-1975\*

Year	Acrea Planted (10 <sup>6</sup> )		Production, bushels (10 <sup>6</sup> )		
	U.S.	North Dakota	U.S.	North Dakota	North Dakota %
1960	1.64	1.28	34.1	26.9	78.9
1961	1.54	1.27	19.0	14.6	76.8
1962	2.42	1.92	71.8	59.6	83.0
1963	1.99	1.65	50.4	43.8	86.9
1964	2.38	2.00	66.7	57.9	86.8
1965	2.23	1.94	69.9	61.1	87.4
1966	2.44	2.08	63.2	55.1	87.2
1967	2.75	2.29	66.4	54.9	82.7
1968	3.56	2.93	99.5	83.4	83.8
1969	3.33	2.78	106.3	91.8	86.4
1970	2.11	1.84	52.8	46.1	87.3
1971	2.86	2.53	91.8	82.1	89.4
1972	2.59	2.33	72.9	65.5	89.8
1973	2.95	2.59	78.5	69.6	88.7
1974	4.07	3.5	79.2	68.8	86.9
1975**	4.57	3.8	120.0	103.7	86.4

\*Data from the Wheat Situation, Economic Research Service, USDA.

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assisted, with funds, the research efforts at NDSU; and the U.S. Durum Growers Association which promotes the interests of the producers and the industry.

#### New Variety Development

As indicated before, one of the prime factors involved in the large scale successful production of durum wheat in North Dakota is the variety development program. Prior to 1960, nine new varieties of durum wheat were developed and released over the 17 year period from 1943 to 1960 (1). Since 1960, eight new varieties have been released cooperatively by the North Dakota Agricultural Experiment Station and the USDA with five of these being released in the past five years (4; Table 5). As a result of this variety development program, North Dakota has not seen a repeat of the serious stem rust epidemics that occurred in the 1950's and 1960's. This accelerated production of new varieties is made possible by the existance of a unique program at NDSU. In the development of a new variety, the time from the first genetic cross to the time of release is approximately 8-10 years. There are basically four stages involved in selecting a new variety that will meet agronomic and quality requirements (5). These stages are listed in Table 6.

Stage 1 is primarily a screening process to eliminate those samples that have little or no potential. The selections that pass, advance to Stage 2. These selections, now called Nursery Samples, are grown in rod-row

Table 3. U.S. macaroni consumption, 1965-1974\*

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1963	1.99	1.65	50.4	43.8	86.9
1964	2.38	2.00	66.7	57.9	86.8
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1972	2.59	2.33	72.9	65.5	89.8
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Stage 1 is primarily a screening process to eliminate those samples that have little or no potential. The selections that pass, advance to Stage 2. These selections, now called Nursery Samples, are grown in rod-row

Table 5. Durum varieties released by North Dakota, 1960-1973\*

Variety	Year Released	Straw Strength	Maturity	Reaction to		Normal Crop <sup>2/</sup>			Overall Quality
				Stem <sup>1/</sup>	Leaf <sup>1/</sup>	Test wt.	Kernel Size		
Crosby	1973	strong	m.early	R	R	avg.	med.	exc.	
Botno	1973	v.strong	early	R	MS	avg.	med.	exc.	
Rugby	1973	v.strong	m.early	R	R	avg.	med.	exc.	
Ward	1972	v.strong	m.early	R	R	avg.	med.	exc.	
Rolette	1971	v.strong	early	R	MS	high	large	good	
Leeds	1966	strong	m.early	R	R	high	med.	exc.	
Wells	1960	m.strong	m.early	R	R	avg.	small	good	
Lakota	1960	m.strong	m.early	R	R	avg.	small	good	

1/ R = resistant; MR = moderately resistant; MS = moderately susceptible.

2/ Avg. = average; med. = medium; exc. = excellent.

\*Source. Reference 4.

Table 6. Four stages in durum cultivar selection

Stage 1	Stage 2	Stage 3	Stage 4
Eliminate poor agronomic types	Eliminate poor agronomic types	Eliminate poor agronomic types	Agronomically acceptable
Eliminate those that do not equal the check variety	Eliminate those that do not equal the check variety	Eliminate those that do not equal the check variety	Equal to check variety
F <sub>2</sub> -F <sub>5</sub> ; 30 g. Kernel size Micro Test Wt. Semolina color Vitreousness Protein Disease	F <sub>6</sub> -F <sub>7</sub> ; 250 g. Micro spaghetti Nursery yield trial test	F <sub>8</sub> -F <sub>10</sub> ; 5 Kg. Continuous spaghetti processing Cooking test Field plot and regional yield trial tests	F <sub>11</sub> Southern increase for possible release F <sub>12</sub> Contract growing for seed increase

yield trials. The amount of seed available at this stage for quality evaluation is, however, limited. After two or three years of nursery tests at several locations within North Dakota, the survivors are advanced to stage 3. At this stage the potential new varieties undergo Field Plot Yield Trial Tests at the following branch stations in North Dakota: Fargo, Langdon, Carrington, Minot, Williston and Dickinson. These selections are also evaluated in regional yield nurseries in North Dakota, Montana, South Dakota, Minnesota and Manitoba. These larger plots provide considerably more wheat for expanded quality testing. Macro-millings are made and the purified semolina processed into spaghetti using a semi-commercial size continuous extruder. Also at this stage a potential variety is required to meet the general criteria that in three consecutive years of testing it must be equal to or better than the existing commercial varieties (6). The selection that passes is then considered for release by the "Variety Release Committee"

at NDSU. After stage 3 an accelerated seed increase program is started on North Dakota experiment stations or in California or Arizona. By this process 5,000 to 10,000 bushels of seed may be produced in 1 year (3) and distributed for further increase to commercial growers in North Dakota with the assistance of the North Dakota County Crop Improvement Association.

Up to this year durum wheat was grown primarily in the northern and north central areas of North Dakota (4). Recent favorable price relationships with other crops has led to a situation in 1975 which sees durum being grown practically all over the state. Thus the estimated high production levels seen in Table 4. The durum variety distribution within the state also has seen some dramatic changes in recent years as a result of the advent of new varieties and the means of increasing them rapidly (Table 7). It is interesting to note the demise of the varieties Leeds and Wells and the upward surge in the use of Rolette and Ward. The varieties Crosby, Botno and Rugby, which were released in 1973, are now beginning to be produced at significant levels.

Table 7. Durum variety distribution, as per cent of crop,  
in North Dakota, 1969-1975\*

Variety	Release Date	1969	1970	1971	1972	1973	1974	1975**
Wells	1960	38.4	29.1	29.8	24.6	27.8	17.5	12
Leeds	1966	60.0	69.3	67.8	67.6	50.0	28.2	15
Rolette	1971				0.5	14.6	38.4	26
Ward	1972					0.8	12.7	32
Crosby	1973							5
Botno	1973							5
Rugby	1973							5
Others		1.6	1.6	2.4	7.3	6.8	3.2	--

\*Data from the Statistical Reporting Service, USDA.

\*\*Estimate.

#### Research at NDSU

In addition to its uniqueness as a major producer of durum wheat, North Dakota also has the distinction of having the only laboratories in the U.S. on a university campus devoted solely to durum quality research. These laboratories are located within the Department of Cereal Chemistry and Technology which was established in 1905 by the North Dakota legislature as a milling and baking facility. In 1938 a special appropriation was made for durum testing equipment, and quality studies began the following year (7). The Agricultural Research Service of the USDA also has a regional Hard Red Spring and Durum Wheat Quality Laboratory based in this department.

Besides collaborating with the Agronomy Department in the new cultivar development program and conducting the annual crop quality survey for North Dakota, this department also conducts research in the following areas:

Development of objective techniques to measure the quality of durum products.

Biochemical research to seek the chemical basis for quality of durum products.

Process research to study the practical problems of the durum processing industry.

Process research to develop new products using durum as a base.

Quality Research. In the durum testing program at NDSU the quality tests used are shown in Table 8. A detailed description of each of these tests is given in the AACC Approved Methods (AACC 1962). However, it does seem appropriate at this time to point out some of the fruits of the quality research program that has occurred over the past few years. For instance spaghetti color and appearance used to be judged visually. Like most subjective evaluations, a certain degree of error is predisposed. Recently

Table 8. Quality Tests used in the durum testing program

Wheat	Semolina	Spaghetti
Test Weight	Protein	Processing properties
1000 Kernel Weight	Ash	Color
Kernel Distribution	Milling Yield	Cooked Weight
Vitreousness	Color	Cooking Loss
Protein	Specks	Firmness

a procedure for measuring spaghetti color by use of a reflectance colorimeter was developed (8,9). In this method the color of spaghetti is expressed in terms of lightness (L%) and yellowness (b%) values, with high values indicating good color (Fig. 1). Another objective method developed was an instrumental measurement of the firmness of cooked spaghetti (10). In this method an Instron Universal Test instrument is fitted with a blunt plastic tooth, so that strands of cooked spaghetti can be sheared. The work (g.cm.) required to shear the spaghetti is used as a measure of cooked spaghetti firmness. More recently a micro unit for producing durum semolina in one operation on sample sizes ranging from 50 to 300 g. has been developed (11). The preliminary results, which will be presented later this month at the AACC Meeting in Kansas City, indicate that this unit can process up to 120 nursery samples per day and that the subsequent speck test on the semolina or the amount of semolina extracted can detect varietal differences.

Processing Research. As was indicated earlier durum wheat is the raw material of choice for producing high quality pasta products. Macaroni products are a good source of many essential nutrients (12; Table 9) but like any single food source it does not supply all the nutrients for a complete human diet. As is typical of most cereals, the protein of macaroni lacks the proper balance of amino acids and is particularly low in lysine. To improve the amino acid balance, other ingredients rich in lysine such as egg solids, milk, dry yeast and soy proteins may be added to semolina and processed into pasta products.

Some recent efforts at NDSU have been concentrated in this area under the sponsorship of the National Wheat Institute and the North Dakota State

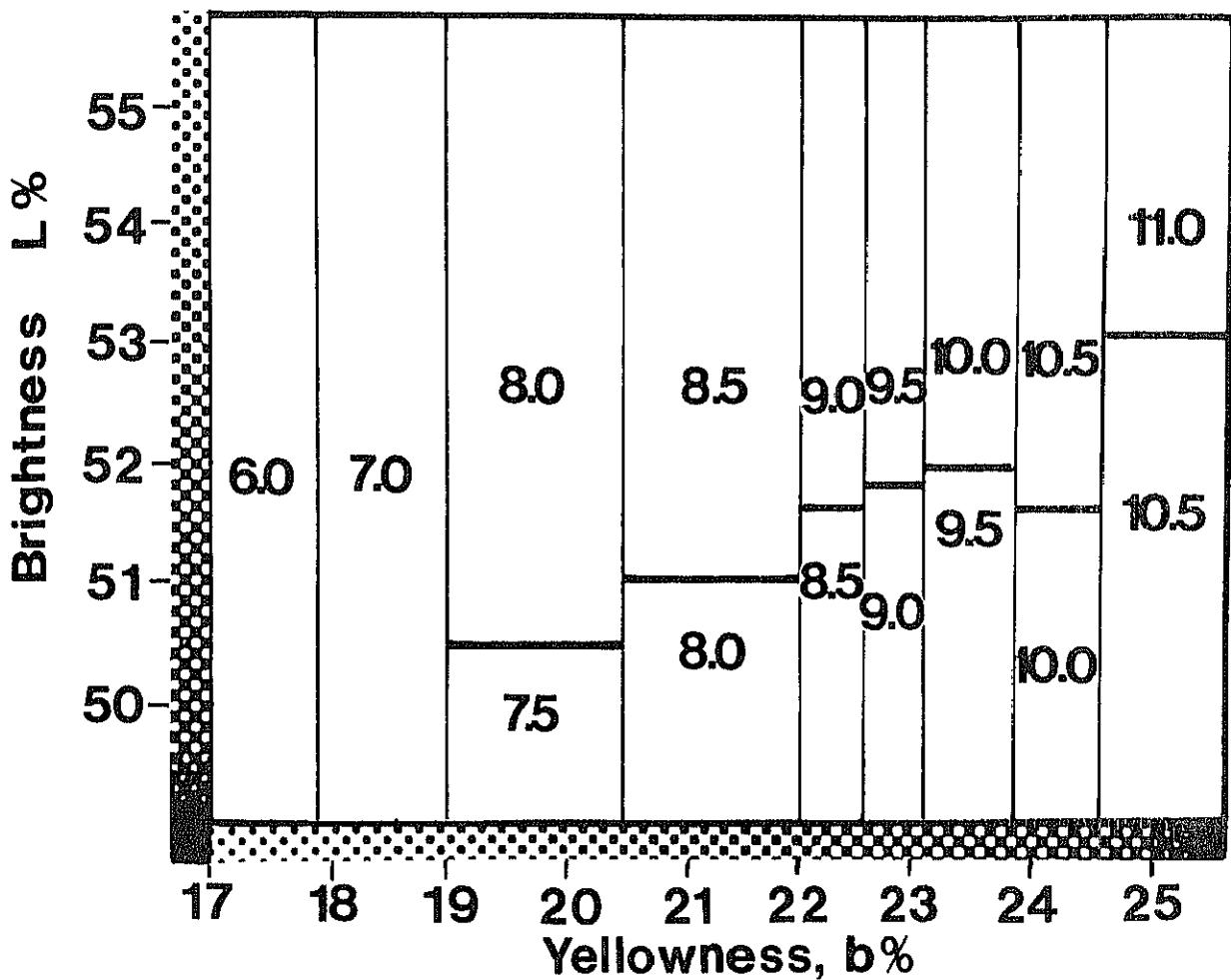


Fig. 1 Color map to convert L% and b% values to spaghetti color scores. This map is used only with Hunter Color Difference Meter reading on continuous processed spaghetti.

Wheat Commission. Results to date have shown that a highly acceptable pasta product can be made from semolina fortified with soy flour, vital wheat gluten and modified whey protein (13). Although the details of this work will not be released until the final report is issued by the Institute probably in June or July of 1976, suffice it to say at this time that both the quantity (20%) and the nutritional quality of the spaghetti protein have been significantly improved. Also a school lunch testing program in Fargo, North Dakota, has shown the product to be highly acceptable. Additional work on the protein enrichment of macaroni products using protein isolates from navy and pinto beans has led to a product which shows considerable promise. The details of this work will be presented by Dr. A. Seyam at the AACC Meeting in Kansas City this month (14).

Finally, a new development in the use of durum semolina has been the preparation of expanded snack foods using milling by-products as ingredients (15). By use of a Wenger X-5 Extruder Cooker, expanded snack foods have been

Table 9. Composition of macaroni and noodles

Food <sup>1/</sup>	Energy Cal per 100 gm	Major Constituents <sup>2/</sup>			
		Protein	Carbo- hydrates	Crude Fiber	Fat
Macaroni:enriched dry	368	12.5	74	0.3	1.2
Macaroni:enriched cooked	107	3.4	23	0.1	0.4
Macaroni:not enriched dry	386	12.5	74	0.3	1.2
Macaroni:not enriched cooked	107	3.4	23	0.1	0.4
Egg noodles:enriched dry	388	15.5	68	0.3	4.4
Egg noodles:enriched cooked	125	4.1	23	0.1	1.5

1/ All products as defined by FDA Definitions and Standards, no optional ingredients included.

2/ All data reported on an as is basis.

prepared which show all indication of high consumer acceptability. With the addition of soy protein isolate, the nutritional aspects of the product is enhanced without any detriment to the snack food quality. Through the judicious use of flavors, a whole range of products can be developed to meet consumer needs.

Conclusion. The research efforts just described are a sampling of current research being done on durum quality and processing. Research continues on variety development and objective testing methods for quality as well as basic research. On this basis the prospects for the continued production and utilization of durum wheat looks very favorable. It is reasonably safe to say that with the continued cooperation between people in research, production, manufacturing, marketing and promotion, durum wheat will continue to enjoy its eminent position as a commodity in the United States.

#### LITERATURE CITED

1. L.D. Sibbitt and K.A. Gilles. Spring wheat varieties:their development, production and utilization. Fifth National Conference on Wheat Utilization Research. Agricultural Research Service. U.S.D.A. 159-170 (1967).
2. M.S. Sjerven. The importance of industry statistics. Macaroni J. 57(5):11-14 (1975).
3. J.S. Quick. Private communication.
4. J.S. Quick and H.D. Wilkins. Durum, a North Dakota specialty. Circular A-361 Rev. Cooperative Extension Service, N.D.S.U., Fargo, ND 58102. June 1975.
5. O.J. Banasik. Durum wheat quality investigations at North Dakota. Macaroni J. 54(2):16-17 (1972).
6. K.A. Gilles. Some new products of durum research. Macaroni J. 56(8): 8-12 (1974).
7. L.D. Sibbitt. Chronological history of cereal technology at N.D.S.U. Macaroni J. 46(1):5 (1962).
8. D.E. Walsh, K.A. Gilles and W.C. Shuey. Color determination of spaghetti by the Tristimulus Method. Cereal Chem. 46:7-13 (1969).
9. D.E. Walsh. Measurement of spaghetti color. Macaroni J. 52(4):20-22 (1970).
10. D.E. Walsh. Measurement of spaghetti firmness. Cereal Science Today. 16:202-205 (1971).
11. S. Vasiljevic, O.J. Banasik and W.C. Shuey. A micro unit for producing durum semolina. A paper to be presented at the 60th Annual Meeting of the AACC, Kansas City, MO. October 26-30, 1975.
12. D.E. Walsh and K.A. Gilles. "Macaroni Products" in Wheat Production and Utilization. Editor G.E. Inglett. The AVI Publishing Co. Inc. Chapter 12, 1974.
13. O.J. Banasik and A.A. Seyam. Private communication.
14. A.A. Seyam, M.D. Breen and O.J. Banasik. Protein isolates from navy and pinto beans. A paper to be presented at the 60th Annual Meeting of the AACC, Kansas City, MO. October 26-30, 1975.
15. M.D. Breen, A.A. Seyam and O.J. Banasik. The influence of milling by-products on the characteristics of expanded snack products. A paper to be presented at the 60th Annual Meeting of the AACC, Kansas City, Mo., October 26-30, 1975.

## CHANGES IN MILLING CHARACTERISTICS OF NEW SPRING WHEAT VARIETIES

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Changes have been observed in the milling performance of wheats in recent years. These changes have been noted in normal height as well as in semidwarf wheats. The assessment of the changes has been difficult because longer extraction, higher ash, and poorer dressed flour is being purchased now than previously, and because of the vast pool of germplasm now used in developing new varieties. Coupled with these factors are new farm management practices and milling processes.

Some changes are due to environment, but the degree and magnitude of environmental effects are obscured by the price and quantity of wheat available. Millers no longer keep large inventories of wheat from which they critically select to maintain uniform mill mixes. The rapid change in wheat lots without proper pretesting has caused unintentional improperly conditioned wheat. Thus, poor milling performance might sometimes be attributed to improper conditioning of wheat prior to milling; however, large scale controlled pilot milled samples have shown definite changes in the milling performance of the wheats over the past several years.

Shuey (1) reviewed some of the changes observed in the milling performance of hard red spring (HRS) wheats and modifications made in the mill flow to overcome these changes. This paper recapitulates some of this earlier work and presents data not previously published.

Two kinds of characteristics are used to appraise the milling characteristics of a wheat sample. The physical characteristics are related to observable phenomena such as vitreousness, mill stream distribution, kernel size, bran size, etc. Chemical characteristics such as the relative level of protein content, fat content, pentosans, etc., are inherent and depend on variety. The two kinds of characteristics may be and often are interrelated, depending on environmental conditions and farm management practices.

One of the first transformations noted in the physical characteristics with the introduction of semidwarf wheats was the appearance of the bran. The cultivars appeared to contain more white caps than previous HRS cultivars. This gave the impression of a softer character, although the starch on bran did not always confirm the higher content associated with soft wheats. Mill adjustments or different conditioning practices did not appreciably alter the appearance of the bran. A discrepancy was also noted between the high flour extraction and seemingly poor cleanup of the bran. This peculiar difference in the bran characteristics was associated with shift in bran size by the change in percent of shorts in the total feed and the shift in type of shorts.

Figure 1 graphically demonstrates the observed percent change of shorts in the feed and the percent of tail shorts for the standard height Chris variety for 4 crop years (1969-72), and for 2 semidwarf cultivars (A & B) for 2 crop years (1971 and 1972). The semidwarf wheats had a greater percentage of shorts in the feed than the standard height variety, yet the percentages of tail shorts of the three varieties were essentially equivalent for the 1971 crop and percentages for the semidwarf were only slightly higher for the 1972 crop. These data showed that there was a definite difference in the distribution of shorts or in the broad characteristics of the bran of the semidwarfs compared to that of the standard height Chris variety. Apparently the broadness of the bran was more difficult to control than before. Although the bran appeared to be unusually friable, the cumulative curve had an ordinary distribution.

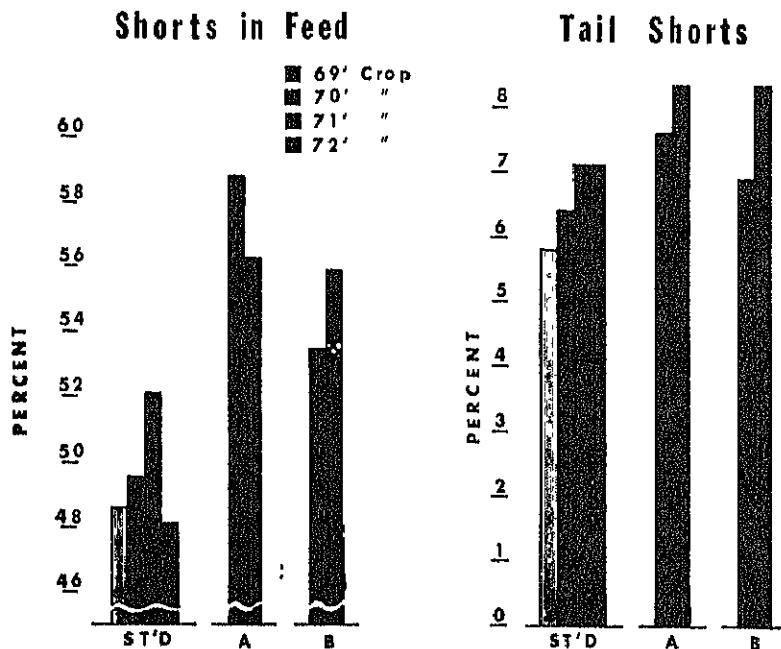


Figure 1. Percent shorts (head and tail) in total feed products and percent extraction of tail shorts.

A shift in the load of the tail-end streams was reflected by a change in the flour percentages of the borderline streams. Table 1 shows the dramatic shift observed in the amount of the low quality flour stream in 1971, 1972, and 1973 compared with amounts for the 1969 and 1970 crop years. Segregation of the wheat samples into conventional height and semidwarf wheats showed approximately 0.5% more low quality flour was obtained from the semidwarf wheats. This suggested that the tail-end mill load for the semidwarf wheats was heavier than for the conventional height wheats, especially since the semidwarfs gave the highest total flour extraction. Another corroborating fact for this belief was that the percent of tail shorts was higher for the semidwarfs; that is, in 1973 the percent of tail shorts was 7.5% for semidwarfs and 6.9% for the conventional height wheats.

Table 1. Percent flour for tail-end streams for 5 crop years (1969-73)

Crop Year	Flour Streams			Total Low Grade & Low Quality %
	5th Break %	Low Grade %	Low Quality %	
1969	1.9	2.5	1.9	4.4
1970	1.9	2.5	1.9	4.4
1971	1.8	3.5	4.2	7.7
1972	1.5	3.2	4.6	7.8
1973	2.0	3.3	4.1	7.4

Not only were the actual percentages changed, but the relation of the streams to each other had changed. Prior to 1971, the quantity of 5th break and low quality flours was the same (Table 1), but after 1970 the low quality flour was approximately 2.5 times greater than the 5th break flour. Also, prior to 1971 the quantity of low grade flour was more than the low quality flour but after 1970 the quantities were reversed. Since some of the new semidwarf varieties had exhibited these types of changes to a degree that exaggerated the differences, the changes were attributed to the genetic characteristics which appeared to have a greater environmental interaction than with the conventional height wheats.

Another notable change was in the relation between starch damage and baking absorption. Historically, a significant correlation has existed between the change in starch damage and the change in baking absorption; as a rule of thumb, a 0.5% increase in starch damage was translated into approximately a 1% increase in baking absorption. Shuey (1) showed that there was a significant correlation between the change in percent starch damage and percent baking absorption for conventional height wheats but essentially little correlation for semidwarf wheats (Table 2). The results demonstrated that the physical properties of the two types of wheat were different and altered the grinding characteristics. The flour particles did not have the same water-binding characteristics even though they had the same relative increase in starch damage.

A chemical factor of great importance for hard red spring wheats has been the protein content. The difference in protein between wheat and flour appeared to be greater for some of the newer varieties of semidwarf wheats than for the conventional height wheats. The data in Table 3 shows that the average difference between wheat and flour protein was 0.5% for the 4 years 1969-72, for the conventional height Chris variety. Chris had a protein difference of 1.0% between wheat and flour for both years 1973 and 1974; the difference for semidwarf Kitt variety averaged 1.2%, but in 1973 the difference was 1.4%.

Table 2. Effect of starch damage on absorption

Type of Variety	Starch Damage <sup>1/</sup>			Bake Absorption <sup>1/</sup>		
	Low %	High %	Ave. %	Low %	High %	Ave. %
<b>Std Height</b>						
Actual	4.66	6.92	5.67	62.5	65.1	63.97
Changes	0.14	1.46	0.87	0.0	2.1	0.61
<b>Semidwarf</b>						
Actual	4.40	9.94	6.49	60.2	66.3	62.94
Changes	0.00	2.07	0.84	0.0	2.0	0.84
Correlation Coefficients: $\Delta$ % Starch Damage, vs.						
$\Delta$ % Bake Absorption						
Standard Height: $r = 0.63$						
Semidwarfs : $r = 0.11$						

1/ 14% Moisture Basis.

Table 3. Wheat to flour protein differences. Averages for North Dakota grown Chris for 4 years

Year	% Extraction	Protein Difference <sup>1/</sup>
1969	79.0%	0.65%
1970	77.5	0.45
1971	78.6	0.45
1972	75.5	0.65

1/ 14% Moisture Basis.

The increased differences between wheat and flour proteins for semidwarfs are demonstrated by the data in Table 4. The protein differences for the two semidwarf cultivars, A and B, followed the same pattern for the 2 crop years. The true difference between the semidwarf cultivars and the Chris variety was at least 0.55% less protein for A, and 0.45% less for B. Thus, a wheat protein of approximately one-half percent more than the amount present in the Chris variety would be required to maintain the same flour protein when the wheats were milled to a constant ash basis. The semidwarfs, Experimentals C and D, did not show as great a difference as Experimentals,

A and B, but all four cultivars showed greater protein difference between wheat and flour than did the Chris variety. The data in Table 5 showed that even greater difference might be expected in future varieties. These experimental varieties showed a protein drop from wheat to flour of 1.5 to 2.7% or from 1.0 to 2.0% less protein than the conventional height check varieties.

Table 4. Wheat to flour protein differences. Comparison of 2 crop years with Chris check

Variety	1971 Crop		1972 Crop	
	$\Delta \text{Wht-Flr}$ <sup>1/</sup>	$\Delta \text{Ex-Ck}$ <sup>1/</sup>	$\Delta \text{Wht-Flr}$ <sup>1/</sup>	$\Delta \text{Ex-Ck}$ <sup>1/</sup>
	%	%	%	%
Chris	0.45	0.00	0.65	0.00
A	1.00	0.55	1.25	0.60
B	0.90	0.45	1.15	0.50
C	-	-	1.05	0.40
D	-	-	0.85	0.20

1/  $\Delta \text{Wht-Flr}$  = difference of wheat and flour protein;  $\Delta \text{Ex-Ck}$  = difference of  $\Delta \text{Wht-Flr}$  between experimental variety and Chris check. (14% moisture basis.)

Table 5. Wheat to flour protein differences. Examples of differences exhibited by some experimental varieties

Check Variety			Experimental Variety			Difference
Wheat Protein %	Flour Protein %	$\Delta \text{Wht-Flr}$ %	Wheat Protein %	Flour Protein %	$\Delta \text{Wht-Flr}$ %	$\Delta \text{Ex-Ck}$ <sup>1/</sup> %
13.6	13.3	0.3	14.2	12.7	1.5	1.2
14.2	13.4	0.8	14.2	11.7	2.5	1.7
14.6	14.0	0.6	11.6	9.5	2.1	1.5
14.9	14.2	0.7	14.9	13.1	1.8	1.1
16.8	16.1	0.7	17.3	15.1	2.2	1.5
16.8	16.1	0.7	16.7	14.0	2.7	2.0
17.2	16.2	1.0	17.5	15.5	2.0	1.0

1/  $\Delta \text{Ex-Ck}$  = difference between experimental variety and Chris check. (14% moisture basis.)

Another phenomenon associated with this protein difference was the peculiar pattern of the cumulative protein curve. A comparison of a normal cumulative protein curve (the Chris variety) and a curve for an experimental semidwarf cultivar are shown in Fig. 2. Apparently some of the oddity of the curve was due either to the distribution of the protein in the kernel and/or the milling characteristics of the wheats. It is unlikely the peculiar shape of the curve was due to distribution or order of the borderline streams as Chris gave a normal curve with the same sequence of streams. Rearrangement of the data according to protein content rather than by ash did not provide a typical cumulative protein curve of normal distribution for the experimental semidwarf like that obtained from Chris.

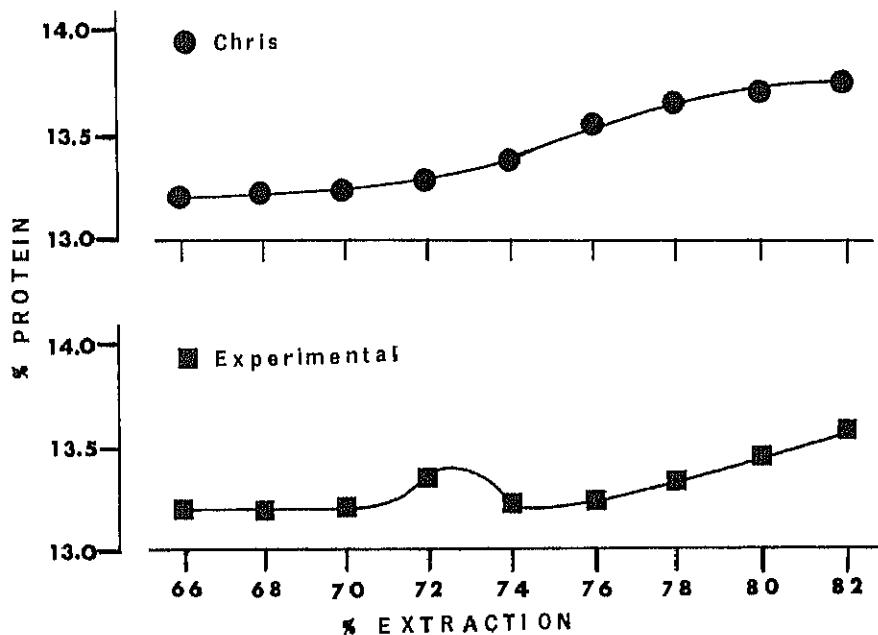


Figure 2. Cumulative protein curve for Chris (conventional height) and experimental (semidwarf) wheats.

A study was initiated to correct the milling problems by changing the mill flow. The Pilot mill described by Shuey and Gilles (2) was reflowed to shift the load of the mill streams back to the head end of the mill. The initial break section of the mill was examined in the greatest detail. The roll settings were not changed and consequently neither was the overall release, but additional and narrower gradations of the 1st and 2nd break stocks were made. The resulting final stream distribution to the reduction rolls was the same but the characteristic of the stock going to the two purifiers was altered. This change, in turn, modified the ground stock going to the two sizing rolls.

The cumulative ash curve showed that there was some improvement in the milling performance for the lower extraction or lower ash flours. There was an approximate 1.5% increase in the extraction for the 0.40% ash patent flour

range, and a 0.5% extraction for the 0.44% ash longer patent flour range, but no advantage for the 0.46% ash flour range. Although there was a small variation in the initial portion of the cumulative curve, the shape of the curve was normal. The improvement might be expected because of the refinements made in the initial grinding stage.

Also, there was a significant change in the tail-end mill stream loads. Previously, the 2nd clear flour was 6.6% extraction; with the new flow, the extraction was 4.9%. Another obvious shift was the ratio between the tail shorts and head shorts. The ratio changed from 1.25:1 to 1:1.

The most noticeable changes due to reflowing the mill were in the cumulative protein curve, and in the difference between wheat and flour protein for the semidwarf wheats. A comparison of the cumulative protein curves before and after reflowing the mill are shown in Fig. 3 for both the conventional height Chris variety, designated "old" and "new" flow, and the semidwarf variety, Era. The two bottom curves for the semidwarf are from the 1972 crop and the 1974 crop, representing the old and new flows, respectively. The data are average figures and even though slight variations were observed for the individual stations, they are representative of the basic patterns. The plateau segment of the curve disappeared with the new flow data and there was a near linear relation between protein content and percent extraction. The conventional height wheat cumulative protein curve for the new flow data was not as linear as for the 1974 semidwarf wheat curve, but definitely smoother than the curve obtained from the old flow data.

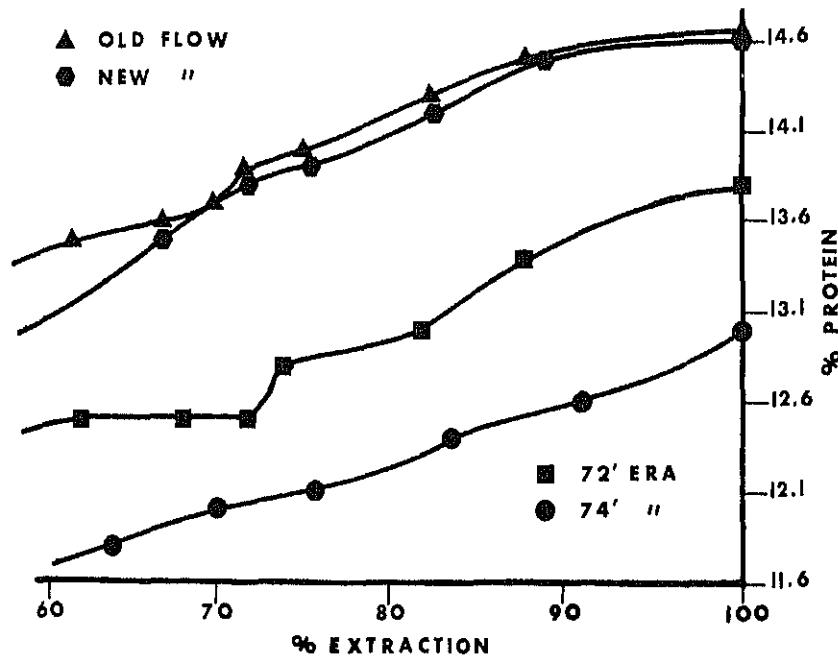


Figure 3. Comparison of cumulative protein curves for old and new flow from conventional height and semidwarf wheats.

There was a greater linear relation between flour protein content and flour extraction for the new flow than for the old flow at 50% extraction or above. The scatter diagrams in Fig. 4 show the relation of flour extraction and calculated protein difference, which is the difference between flour protein at 100% extraction and the flour protein for the given extraction. The protein difference was calculated to eliminate the variance in initial protein content of the samples. A correlation coefficient of 0.56 was found between the calculated protein difference and percent extraction for the old flow data, while a correlation coefficient of 0.72 was found for the new flow data. The lower correlation might be expected for the old flow data since the relation between protein and extraction has traditionally been curvilinear.

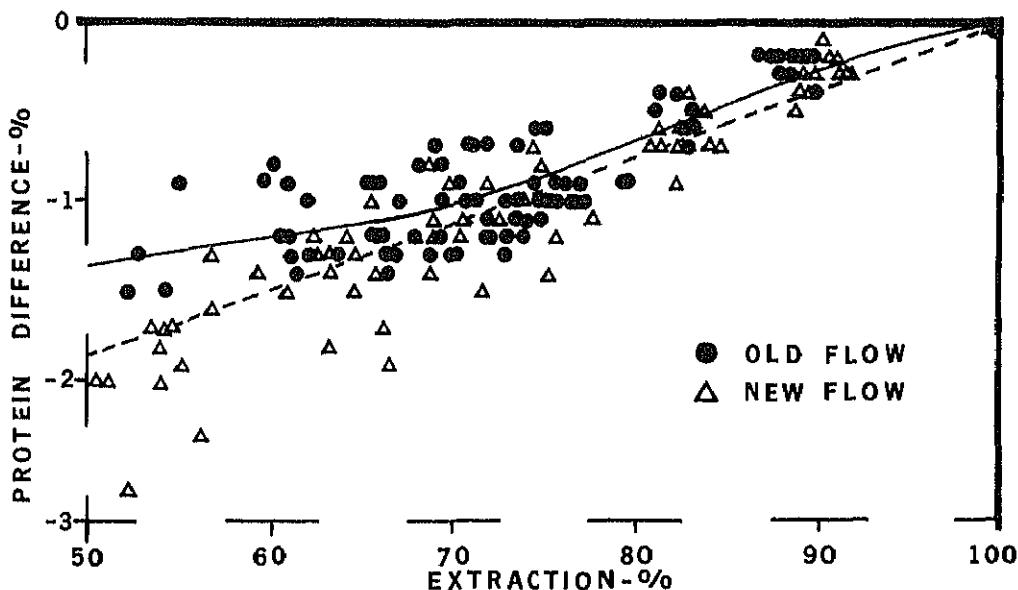


Figure 4. Calculated protein differences of flour at given extractions from whole wheat.

The shift in mill stream loads, the nature of the starch damage, and the abnormal cumulative protein curves as well as other observations indicated a definite modification of hard red spring wheat response to the historically accepted milling practices. These changes may be attributed to several factors such as new germplasm, farm management practices, greater response of varieties to environmental conditions, slight alterations in milling practices, etc. Reflowing the mill with finer gradation of the stock in the head end of the mill suggested that many of the peculiarities could be overcome or minimized. One of the most striking effects in reflowing the mill was the linear response of flour protein to percent extraction.

Literature Cited

1. Shuey, W. C. Observations on milling characteristics of commercial and experimental spring wheats. Wheat Quality Conference Report, p. 5 (1975).
2. Shuey, W. C., and Gilles, K. A. Laboratory scale commercial mill. Operative Millers Tech. Bul., p. 3100 (1969).

## WHEAT PEARLING

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### Introduction

Wheat in a whole kernel form is a relatively unexploited food use. Bulgur (1), a parboiled, partially debranned wheat, is an exception, though most bulgur is normally cracked to grits to avoid the long cooking time and toughness of whole kernel bulgur.

An excellent whole grain wheat product known as WURLD Wheat has been developed by Morgan and co-workers (2) of the U. S. Department of Agriculture. In this process, wheat is given a hot soak that causes the kernel to open up and expose the crease bran. At this point, the drained wheat is treated with strong alkali and steam which causes the bran to loosen and to slough off when subjected to high turbulence in water. The small residual alkali on the kernels is neutralized with an acetic acid rinse giving an attractive, yellowish-white whole kernel product of about 85% yield. The product is excellent, but the wet process adds drying expenses and yields a wet, alkaline bran residue that represents a disposal problem. The product is not commercial.

Another approach to a whole kernel product is dry mechanical debranning or pearling. There are a number of articles and patents that discuss wheat peeling, many from the standpoint as a pretreatment to the milling of white flour. Grosh and co-workers (3) found that removal of 6 to 11% of the wheat kernel in a Strong Scott Laboratory Pearler under several different conditions resulted in a 1 to 6% lower flour extraction rate and flours had slightly higher ash contents. They surmised that damage to the inner bran in the form of scratches and fractures caused it to powder more easily on milling. Accordingly, the wheat had to be milled more gently.

Grosh and co-workers (3) also found that wheat treated with 5% water 5 minutes before scouring in a modified Forster Model 10 Scourer had outer pericarp (beeswing) well removed (except in the crease) but resulting flour yields on milling were not improved and ash contents of the flours increased slightly.

Pomeranz (4), in reviewing wheat peeling in 1961, concluded that none of the various milling pretreatments such as dry peeling, wet peeling or chemical peeling, had resulted in higher flour yields or comparable flour quality.

Earle (5, 6) studied dry peeling in a stripper based on the design of standard rice mills. Five percent water was added to the wheat a few minutes before stripping. The stripper was kept full so that pressure was created within. According to Earle, this caused the inner bran layers to be compacted and toughened while rubbing off the water loosened outer pericarp or beeswing (80% removal, crease beeswing not removed). When such bran-toughened, peeled wheat was milled for white flour, he found 1 or 2% better total flour extraction, several percent better patent flour extraction, and better quality flour

in terms of lower ash content. He noted, too, that the resulting bran was more wholesome (less insect and mold fragments, less dirt), less bitter and more sweet in flavor, and less scratchy in mouth feel, indicating greater appeal for food uses. He further noted that such peeled wheat makes a more desirable whole wheat type flour for the same reasons.

In 1959, one half of one percent of all flour consumed in the U.S. was whole wheat flour (7). By 1966 this had increased to 4% and in 1973 the figure was 6%. Siegel, food industry analyst at Rothchild and Co. (8), notes that high fiber breakfast cereals such as bran flakes and all bran are benefiting in the current market from publicity regarding nutritional and health value. It seems to us that wheat millers and processors might consider development of an upgraded whole wheat flour or bran products along the lines of Earle's work, that is, whole wheat flour and bran products with the beesswing removed. To the extent that wheat washers (9) followed by scourers are still used today, the whole wheat flours and bran products from such mills represent a step in this direction.

A pearled wheat known as Rycena has been produced in Australia primarily for export to Asian countries as a rice substitute. An improved version of Rycena was developed a few years ago that entailed fortification with a synthetic extruded kernel containing lysine, vitamins and minerals along with a substance reputed to neutralize the wheat flavor of Rycena. We have not found any literature references to these products, however.

A quick cooking pearled wheat has been described by Lewis and coworkers (10) in which pearled wheat is impregnated with a saturated salt solution, then partially cooked and partially dried. In one example, 44% of the weight of the wheat was taken up. After pasteurization, the product is stable and resistant to attack by vermin and microorganisms. Cooking in a large volume of water (6 volumes) reduces the salt content to a palatable level. Cooking time is about 10 minutes. Hunt Wesson Foods, Inc. is currently evaluating such products for possible commercialization.

Wasserman and coworkers (11, 12) at the USDA's Western Regional Research Center have studied the operating variables of laboratory Engleberg and McGill rice mills and a CeCoCo Barley and Rice Polishing Mill in debranning wheat. The Engleberg and McGill mills achieve debranning by causing the wheat grains to rub against each other. Tempering wheat up to 13.7% moisture, addition of up to 3% water and 1% abrasive  $\text{CaCO}_3$  a few minutes before milling improved the rate and degree of fiber reduction while optimizing the yield of debranned wheat. Debranning in this type of mill is best achieved by multiple passes. On an HRW wheat, 85% yield of wheat at 0.8% fiber was achieved.

In the CeCoCo, wheat is debranned by rubbing of the grain against an abrasive carborundum surface. Higher feed rates to the CeCoCo, tempering wheat up to 14.6% moisture, and addition of up to 3% water a few minutes before pearling improved rate and degree of fiber reduction while optimizing yield of debranned wheat. On a soft white wheat, 80% yield of debranned wheat with 1.4% fiber was achieved in two passes. Wasserman and coworkers (12) concluded that it was possible to reach lower fiber contents in the mills that rub grains one against another such as the Engleberg or McGill mills, but the abrasive pearlers such

as the CeCoCo have greater capacity, greater choice of equipment and no need for  $\text{CaCO}_3$  abrasive.

### Experimental

Our work with pearled wheat was undertaken to characterize certain aspects of composition and use. To accomplish debranning, we used a CeCoCo Barley and Rice Polishing mill. The machine has an abrasive carborundum cone which rotates on a horizontal axis inside a slotted screen. Gaines variety, soft white wheat at 10% moisture was sprayed with 2% water and allowed to temper 5 minutes and then passed through the CeCoCo mill with feed gate width at 1/2 inch, stone to screen gap of 1/2 inch and minimum pressure on the exit gate. The first pass wheat as well as the abraded bran was collected and weight yield determined. The first pass wheat (without additional water) was then subjected to a second debranning pass and so forth until a total of 6 passes were completed.

### Results

The composition of the original wheat, and the composition and yield of the pearled wheat after from 1 to 6 passes in the CeCoCo mill, are presented in Table 1. The composition of the brans abraded off are shown in Table 2.

Table 1. Composition of wheat and CeCoCo mill debranned wheat; dry basis; average of duplicate determinations; 2% water sprayed on before step 1 milling, dry milling in other steps

Composition <sup>1/</sup>	Original Wheat	After Step 1	After Step 2	After Step 3	After Step 4	After Step 5	After Step 6
Crude fiber, %	3.0	1.6	1.4	1.2	1.3	1.1	1.1
Thiamine, $\gamma/\text{g.}$	3.4	2.9	2.6	1.9	1.7	1.5	1.1
Crude fat, %	1.6	1.5	1.4	1.3	1.1	1.2	1.0
Phytate phosphorus, %	0.20	0.17	0.15	0.14	0.13	0.12	0.13
Ash, %	1.71	1.48	1.27	1.26	1.19	1.20	1.11
Total phosphorus, %	0.28	0.26	0.25	0.21	0.20	0.20	0.19
Lysine <sup>2/</sup> , g./16 g. N	2.84	2.62	2.53	2.64	2.55	2.68	2.40
Protein (N X 5.7), %	13.7	13.6	13.1	13.1	12.5	12.1	11.8
Glutamic acid <sup>2/</sup> , g./16 g. N	33.3	34.3	34.6	34.4	35.8	36.1	35.0
Moisture <sup>3/</sup> , %	10.0	10.4	10.3	10.2	10.2	10.1	10.1
Yield, % of original wheat	100	89	80	73	66	60	54

<sup>1/</sup> Listed in order of decreasing milling effect.

<sup>2/</sup> Single analysis.

<sup>3/</sup> As is basis.

Table 2. Composition of bran abraded from wheat by a CeCoCo mill; dry basis; average of duplicate determinations; 2% water sprayed on before step 1 milling, dry milling in other steps

Composition	Original Wheat	After Step 1	After Step 2	After Step 3	After Step 4	After Step 5	After Step 6
Crude fiber, %	3.0	8.8	3.5	2.2	1.8	1.6	1.4
Thiamine, $\gamma/g.$	3.4	6.0	8.1	7.5	7.4	6.2	4.6
Crude fat, %	1.6	3.5	3.2	3.0	2.9	2.3	2.0
Phytate phosphorus, %	0.20	0.34	0.31	0.27	0.24	0.21	0.20
Ash, %	1.71	3.41	2.96	2.28	2.06	1.75	1.66
Total phosphorus, %	0.28	0.58	0.54	0.44	0.43	0.34	0.27
Lysine <sup>1/</sup> , g./16 g. N	2.84	3.38	3.21	3.17	2.81	2.50	2.61
Protein (N X 5.7), %	13.7	15.7	17.5	18.0	16.2	15.2	14.3
Glutamic acid <sup>1/</sup> , g./16 g. N	33.3	27.9	31.1	31.4	32.5	33.0	34.0
Moisture <sup>2/</sup> , %	10.0	13.5	10.0	9.8	9.7	9.5	9.6
Yield, % of original wheat removed each pass	--	11	9	7	7	6	6

<sup>1/</sup> Single analysis.

<sup>2/</sup> As is basis.

Figure 1 shows graphically how composition of the wheat changed with increasing passes through the CeCoCo mill. Total phosphorous and phytate phosphorous followed curves similar to the one for ash content.

In vitro protein digestibilities and dry matter digestibilities, determined by the method of Saunders and Kohler (13), of the bran fractions from the first four passes are presented in Table 3. Also included in Table 3, for comparative purposes, are data on wheat and products of conventional flour milling (13).

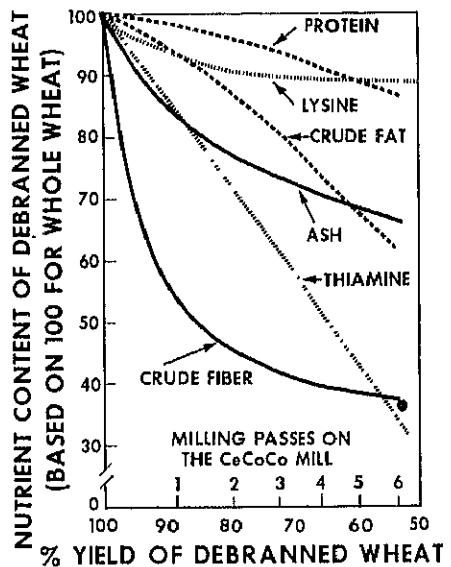
Differences in tenderness (shear resistance), as determined on a Lee Kramer Shear Press, and water absorption, relative to cooking time, for pearled wheats, bulgur, and rice, are shown in Figure 2.

Cooking completeness was also determined by the disappearance of white kernel centers. Using this criterion, pearled wheats were completely cooked in 25 to 30 minutes.

Solids lost in the cooking water ranged from 4 to 5% for all pearled wheat samples when cooked 20 to 30 minutes. Composition, dry basis, of lost solids

from first pass pearled wheat was protein 10.6%, crude fat 0.6%, and ash 6.7%; for sixth pass pearled wheat: protein 6.7%, crude fat 0.3%, and ash 3.8%. Whole wheat cooked for 1 hour lost about 3% solids and bulgur cooked for 30 minutes (still incompletely cooked) lost 2.7% solids. After 20 minutes cooking, Calrose rice had lost 6.7% of its solids to the cooking water. Longer cooking of the rice resulted in excessive water absorption and pasty surfaces.

Figure 1. Change in composition of debranned wheat with degree of CeCoCo milling; 2% water sprayed on wheat 5 minutes before first milling pass; dry milling in other passes.



For storage studies, Gaines white wheat was milled in 4 passes on the CeCoCo to give a yield of 78% of pearled wheat of 1.2% fiber, dry basis. Samples were stored at 10 and 13% moisture in sealed containers at 0°, 90°, and 100°F. for up to 8 months. The 11 member odor panel testing the raw pearled wheats did not indicate any major differences between the control (0°F., 10% moisture) and samples stored under other conditions.

Thiobarbituric acid values (14) corroborated a lack of oxidative rancidity in all samples throughout storage. Values (absorbance at 532 nm.) did not increase with storage time; they generally ranged from 0.01 to 0.05. Caldwell and Grogg (14) found that an oat cereal invariably smelled rancid at an absorbance of 0.25, or higher; oatmeal cookies at 0.55, and higher. The threshold value for pearled wheat is not known but, apparently, was not approached in 8 months storage under the conditions of this experiment.

Lipid acidities (15) were stable or increased slightly (by a maximum of 25%) for the 10% moisture samples. In the 13% moisture samples held at 90° or

100°F., lipid acidities increased from 27  $\mu$ eq. per g. of sample to 44 and 60  $\mu$ eq. per g. of sample respectively at 8 months. For comparative purposes, a 13% moisture straight grade flour stored at 100°F. in a sealed container increased in lipid acidity from 8  $\mu$ eq. per g. flour to 18 at the end of 6 months (15).

Table 3. In vitro protein digestibilities (PD) and dry matter digestibilities (DMD) of bran abraded from wheat compared to products from the roller milling process and whole wheat

Product	PD, %	DMD, %
Bran from pearled wheat <sup>1/</sup>		
First pass	93.7	65.2
Second pass	95.9	82.8
Third pass	96.8	87.9
Fourth pass	96.2	89.5
Roller mill products		
Bran	71.5	34.8
Shorts	79.1	48.4
Red dog	89.5	73.1
Flour	96.7	91.3
Whole Wheat, Wiley mill 20 mesh	91.0	85.0

<sup>1/</sup>Average of duplicate determinations.

For flavor testing by the 21 member panel, 100 g. of pearled wheat was added to 500 ml. boiling water containing 2 g. of salt, returned to a boil and simmered 25 minutes at which time it was drained in a sieve. The triangle test, duplicated, was used. Significant differences ( $P \leq 0.05$ ) in flavor developed between 6 and 8 months storage for samples held at 100°F. In the 13% moisture materials, there was a significant preference (67%) for the 0°F. sample by those who correctly identified the odd sample in the triangle. In the 10% moisture materials, there was no significant preference at 8 months, though more judges were inclined to favor the 100°F. sample. Overall, it appears that no gross flavor deterioration occurred either at 90° or 100°F. for 6 to 8 months, even though some significant flavor changes did occur.

#### Discussion

The objective of debranning wheat in the present work was to produce a storage stable, high yield product in whole kernel form, one that could be cooked like rice in a relatively short time and have desirable texture and flavor. Results of odor, thiobarbituric acid, lipid acidity and flavor tests indicate that moderately debranned wheat (78% yield) has good storage stability, particularly at moisture contents below 13%. On the other hand, Boles and Ernst (16) evaluated samples of our pearled wheat and found them to be preferentially attractive to adults of the rice weevil and the red flour beetle; they also

stimulated reproduction of these insects to a greater extent than WURLD Wheat cracked bulgur, Gaines variety whole wheat, or white flour.

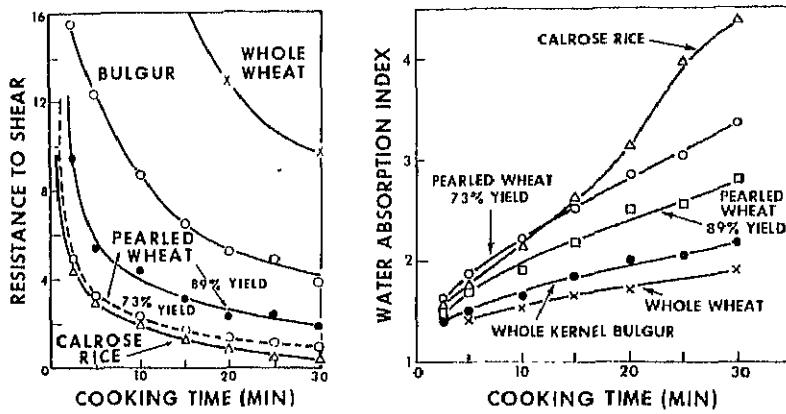


Figure 2. Tenderness (shear resistance; Lee Kramer Shear Press) and water absorption index of wheat and rice products. 100 g. of product were placed in 500 ml. boiling water, returned to a boil and simmered.

From the standpoint of fiber reduction or ash reduction, CeCoCo mill pearling is decidedly inefficient compared to roller milling. For example, crude fiber contents of 75, 85, 90, 95, and 100% extraction flours from roller milling are 0.15, 0.30, 0.80, 1.40 and 2.00% respectively, compared with the higher crude fiber contents for pearled wheats (Table 1) of similar extraction (yield).

This inefficiency is explained, in part, by the crease bran (20 to 30% of total) which is not accessible to the abrasive action. Additionally, most of the starting wheat kernels tended to have a triangular cross section (Figure 3) with the result that the points of the triangle were heavily abraded (dorsal ridge and two cheeks), but the areas between the points were relatively lightly abraded. An examination of the pearled wheats showed that a portion of the germ (embryo and scutellum) remains. A progressive loss of thiamine with increased pearling (Table 1) indicates a gradual loss of germ (scutellum contains 62% of all thiamine in the wheat kernel).

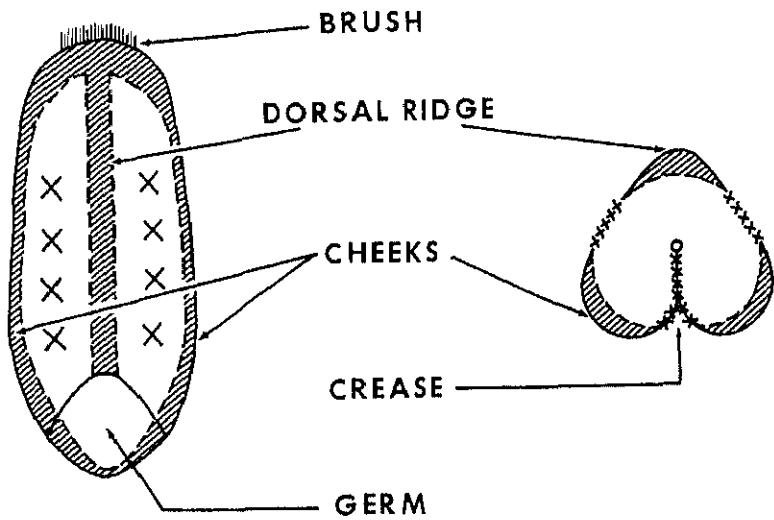


Figure 3. Location of heaviest abrasive action (hatched area) on Gaines soft white wheat during pearling in the CeCoGo mill. X's represent areas where residual bran is usually present including bran layers out to and including cross cells. 73% hypothetical yield.

The epidermis and hypodermis (beeswing) were the only tissues essentially completely removed (except in the crease). Addition of water at the 2% level, 5 minutes before pearling, aided substantially in removing the beeswing. The beeswing has been determined to be about 4% of the kernel weight; its crude fiber content is 24% (17). If whole wheat contains 2 to 3% crude fiber, then one third to one half of the crude fiber is located in the beeswing and, thus, its removal contributes substantially to fiber reduction in pearled wheats.

Ash reduction in pearled wheat was considerably less than for roller mill flours of similar extraction. Since some 60% of the total ash of the wheat kernel resides in the aleurone, this is evidence of substantial retention of aleurone tissue in the pearled wheats.

\* Reduction in lysine content from whole wheat to 75% extraction flour (roller mill) averages about 29% (2.60 g. down to 1.94 g. lysine per 16 g. N). At comparable yield, lysine reduction in pearled wheat was only about 10%. Again, this reflects retention of germ and bran tissue at certain locations in the pearled wheat.

The compositions of abraded brans (Table 2) indicate that they have substantial nutritional value. Whereas the protein of the bran from roller milling is only partially digestible by monogastric animals (Table 3), the protein of brans produced by abrasive action in the CeCoCo mill showed higher *in vitro* protein digestibilities; these are high even after taking into account the higher starchy endosperm content of abraded brans. Saunders *et al.* (18) showed that low protein digestibility in roller milled brans is, in part, due to a physical barrier, the intact aleurone cell wall which prevents access to the aleurone cell contents by the digestive enzymes. Microscopic examination of pearled wheat showed that from the cross cells inward, the abrasive action was progressive, cutting-up and, generally, disrupting the cellular structure of successive layers. This, then, probably explains the high *in vitro* protein digestibilities of the abraded brans.

The presence of considerable starchy endosperm in the abraded brans, the high protein digestibilities, the substantial content of nutrients, and the floury (small particle size) nature of the brans suggest usefulness in food formulations. The quality of the brans might be enhanced if the beeswing were removed before pearling. This can be accomplished, in large part, in mills such as the McGill of Engelberg (5, 11).

In conclusion, pearled wheats were produced having cooking tenderness approaching that of rice. Cooking with an excess of water was preferred to minimize grain stickiness. However, this had the disadvantage of a 4 to 5% loss of solids in the cooking water.

In general, before wheat can be consumed, it must be processed and cooked. Many of the available processes and cooking methods for wheat are quite complex and do not readily lend themselves to small village operations. Abrasive pearling can be carried out easily on a small scale and the pearled wheat product lends itself to the easiest of cooking methods, boiling in water. In addition, the bran products obtained in the pearling process may have direct application as food material, for example, in a whole wheat-type chapatti. Such a total food use system for wheat would require appropriate wheat cleaning equipment. Additionally, the palatability of the bran fractions would probably be substantially enhanced if a pre-pearling or scouring operation to remove beeswing was incorporated in the overall process.

#### Literature Cited

1. Haley, W. L. and Pence, J. W. Bulgur, an ancient wheat food. *Cereal Sci. Today* 5: 203-207, 214 (1960).
2. Morgan, A. I., Barta, E. J., and Graham, R. P. Chemical peeling of grain. *Chem. Engr. Symp. Series* 62(69): 138-141 (1966).
3. Grosh, G. M., Shellenberger, J. A., and Farrell, E. P. Milling properties of wheat in relation to pearling, scouring and impaction. *Cereal Chem.* 37: 593-602 (1960).

4. Pomeranz, Y. Peeling of wheat kernels. *Cereal Sci. Today* 6(2): 76-77, 79 (1961).
5. Earle, T. Compaction tempering - An innovation in the milling industry. *Northwestern Miller*. April 14, 1959.
6. Earle, T. Method of stripping epidermal materials from grains. U.S. Patent 2,867,256 (Jan. 6, 1959).
7. Anon. Many new flour consumption insights. *Milling and Baking News* 54 (28): 7 (Aug. 26, 1975). Based on an Economics Research Service, USDA report.
8. Anon. Impressed by growth in cereal market. *Milling and Baking News* 54 (29): 8 (Sept. 2, 1975).
9. Ofner, F. R. The wheat washer problems and solutions. *Assoc. of Operative Millers Bulletin*, April 1956, page 2238.
10. Lewis, D. A., Lewis, V. M., and Lewis, J. M. Quick cooking foodstuffs. U.S. Patent 3,495,989 (Feb. 17, 1970).
11. Wasserman, T., Ferrel, R. E., and Pence, J. W. Mechanical debranning of whole kernel wheat. I. Engleberg and McGill mills. *Cereal Sci. Today* 15(5): 134-136, 138, 139 (1970).
12. Wasserman, T., Mossman, A. P., and Fellers, D. A. Mechanical debranning of whole kernel wheat. II. CeCoCo pearling mill. *Cereal Sci. Today* 17(3): 82-85, 92 (1972).
13. Saunders, R. M. and Kohler, G. O. Invitro determination of protein digestibilities in wheat millfeed for monogastric animals. *Cereal Chem.* 49: 98-103 (1972).
14. Caldwell, E. F. and Grogg, B. Application of thiobarbituric acid test to cereal and baked products. *Food Technol.* 9(4): 185-186 (1955).
15. Mecham, D. K. and Mossman, A. P. Titratable acidity in water saturated n-butyl alcohol and petroleum ether extracts of some stored wheat products. *Cereal Chem.* 51: 478-487 (1974).
16. Boles, H. P. and Ernst, R. L. Reaction of stored product insects to some newly developed whole wheat products. Rep't. 6th Nat'l. Conf. on Wheat Util. Research. ARS 74-54 (USDA), August 1970.
17. Pomeranz, Y. Wheat Chemistry and Technology. Am. Assoc. of Cereal Chem., St. Paul, MN., page 70 (1969).  
Saunders, R. M., Walker, H. G., and Kohler, G. O. Aleurone cells and the digestibility of wheat millfeeds. *Poultry Sci.* 48: 1497-1503 (1969).

## UPGRADING CEREAL STRAWS

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Historically in the United States, cereal straws and stovers have been neglected as feedstuffs because of an abundance of inexpensive high quality roughage and cheap grain. Recently, however, since prices of forages and feed grains have increased sharply, livestock feeders have been looking with renewed interest at alternative low cost feedstuffs. Interest in feeding straws has developed particularly in areas where open field burning is the traditional straw disposal methods. Pressure from legislators and residents of urban areas threatens to ban open field burning so that development of alternative disposal methods has become imperative. Disposal through feed use is one of the more attractive possibilities.

Table 1 shows the acreages of the various cereal actually harvested for grain

Table 1. Cereal acreage harvested for grain, USA, 1973

Cereal	Acres $\times 10^6$
Wheat	53.9
Oats	14.1
Barley	10.5
Rice	2.2
Rye	1.0
	81.7
Corn	61.7
Sorghum	15.9
	77.6
	159.3

in 1973 (1). If we say conservatively that one ton of residue is produced per acre, we arrive at a figure of 159.3 million tons of cellulosic waste that is potentially available for feed use. This amounts to more than the hay crop of 133 million tons harvested in the same year. These cereal wastes that are ordinarily left in the field contain at least as much energy as that of the harvested grain crop, and this additional feed energy can be obtained with the expenditure of very little additional petrochemical energy. The feed energy from this source can be used by ruminants only, and is not involved in the man versus animal competition for energy from cereal grains.

It is fairly evident that cereal residues left in the field are not good feeds because they are not used for this purpose except in periods of extreme economic stress. Table 2 contrasts the composition of rice straw with that of a

Table 2. Composition of rice straw and alfalfa hay

Component	Rice straw, %	Alfalfa, <sup>1</sup> %
Protein (Nx6.25)	4.0	15.5
Lignin	5.7	10.5
Total ash	17.4	9.1
Silica	16.5	--
Ether extract	2.4	2.6
Cellulose	35.9	35.0
Hemicellulose	17.4	8.1

<sup>1</sup>Binger et al (1961).

good ruminant grade alfalfa hay (2). The most striking nutritional difference is in the protein content--that of alfalfa is high enough to permit its use as a complete feed; that of rice straw is too low to maintain an animal without protein supplementation. An animal cannot physically consume enough low protein straw to meet its daily protein requirement. Lignin, a structural material which interferes with digestibility, is higher in alfalfa than in straw. This is offset by the fact that rice straw contains over 16% of silica which also impedes digestion (3). Other cereal straws have less silica but more lignin (up to 10%). Rice straw's main asset is that it is rich in cellulose and hemicellulose. Under suitable conditions, these can be digested by rumen microorganisms to provide energy for the host animal.

The problem is that digestive enzymes of the ruminal microorganisms are usually unable to get at the cellulose and hemicellulose to break them down to simple carbohydrates which are finally converted to energy-rich volatile fatty acids. Some form of processing is necessary to improve the digestibility of carbohydrates of straws to permit their extensive use in animal feeds. The question is "how much" and "what kind" of processing to carry out. Proposed processes to upgrade cereal straws range from simple grinding treatments to elaborate chemical pulping operations, with most workers investigating treatment conditions somewhere between these two extremes.

In the 1960's, a group at the University of Nottingham showed that when untreated ground barley straw was included in steer diets at levels of up to 30% of the total diet, feeding results were just as good as those obtained with all concentrate rations (4). Table 3 summarizes this work. As expected,

Table 3. Summary of University of Nottingham<sup>1</sup> - Cattle trials

Barley straw, % operation	Daily gain, lb	Daily feed, lb	Feed/gain
0	2.84	20.1	7.1
10	2.92	19.5	6.7
30	3.17	23.4	7.4
50	2.78	22.2	8.2
70	2.24	23.9	10.7

<sup>1</sup>Swan *et al.* (1966, 1967, 1970).

poorer results were obtained at the 50 and 70% levels. The straw must be milled through a screen not larger than 1/2", and adequate nitrogen must be present to promote rapid bacterial growth. Other English workers were able to duplicate this work with barley straw milled through a 1" screen (5). Gains and feed efficiency were lower at the 20 and 30% levels but not significantly so. In this country T. W. White and co-workers in Louisiana achieved similar results feeding steers rice straw milled through a 1/2" screen when straw levels did not exceed 20% (6). Recent work with lambs at the University of Washington indicates that ground wheat straw can replace 25% of alfalfa hay in an all roughage ration without causing a significant performance decrease (7). All these workers used ground straw; no one has successfully fed long or coarsely chopped straw. Workers at the Hopland Field Station of the University of California fed diets containing ground (5/16" screen) pelleted corn stalks, rice straw or barley straw in a digestion trial (8). All materials were supplemented with nitrogen, phosphorous, and sulfur prior to pelletizing. The results of the trial indicated that properly supplemented corn stalks or rice straw could be used as part or all of a sheep maintenance ration; barley straw was inadequate for this use.

At the other extreme of processing, Kellner, in 1900, prepared a highly pulped product by cooking rye straw for 3-1/2 hr at about 100 psi in a solution containing sodium hydroxide, sodium carbonate, sodium sulfite, and sodium thiosulfate (9). The washed and dried material had an organic matter digestibility of 88.3%. About 50 years later, Woodman and Evans, working with a similar product from wheat straw, reported that it had the energy equivalent of oat grain in digestion trials (10). But even at today's feed and roughage prices, production of these highly pulped products for animal feeds cannot be justified economically.

Fortunately the digestibility of cereal residues can be increased appreciably without recourse to such vigorous chemical procedures. Most of the successful treatments involve use of sodium hydroxide or other alkaline materials. In laboratory experiments with ground corn cobs Chandra and Jackson investigated the use of ten common delignifying reagents as shown in Table 4 (11). Only

Table 4. Pulping reagents used in corn cobs

Reagents <sup>1</sup>	Concentrate range g/100 g roughage
Na <sub>2</sub> S	0-15% in H <sub>2</sub> O
Na <sub>2</sub> SO <sub>3</sub>	"
NaOH	"
Na <sub>2</sub> CO <sub>3</sub>	"
NaOH+Na <sub>2</sub> CO <sub>3</sub>	"
Ca(OC <sub>1</sub> ) <sub>2</sub>	"
H <sub>2</sub> O <sub>2</sub>	"
NaOH+C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>	100%
CH <sub>3</sub> OH	"
C <sub>6</sub> H <sub>5</sub> OH	"

<sup>1</sup>Chandra and Jackson (1971).

sodium hydroxide gave good digestibility increases at reasonable levels. Wilson and Pigden had previously demonstrated that the digestibility of wheat straw increases linearly as the amount of sodium hydroxide is raised to about the 9% level, but further increases had no additional effect (12) (Figure 1). A similar effect was noted for poplar wood.

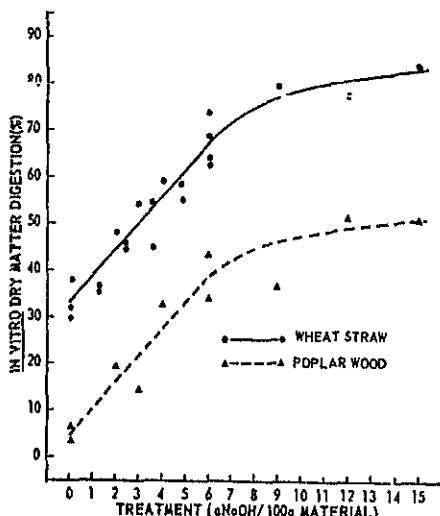


FIG. 1. The effect of sodium hydroxide upon the percentage dry matter digestion *in vitro* with rumen microorganisms. Each point represents the mean of three tubes. The curves were fitted by eye.

The reason that alkali increases digestibility of straw-like materials is not entirely clear. Tarkow and Feist (13) suggest that the major factor is that crosslinking ester linkages in the hemicellulose fraction are saponified, opening up the structure for enzymatic attack. Other changes also take place. Acetyl groups are removed from the hemicellulose fraction and some swelling and

loss of crystallinity of cellulose occurs. A portion of the encrusting silica is solubilized by alkali (14,15). Although a few lignin-carbohydrate linkages may be disrupted, it seems unlikely that it is necessary to break-up much lignin to get an acceptable digestibility increase with hardwoods and straws (16). These increases can be achieved under mild reaction conditions that do not lead to degradation of lignin. Chemical analyses for lignin in treated and untreated straws usually show very small differences as shown in Table 5 (17), but digestibility changes are easily detected. Most investigators have used sodium hydroxide because of its availability and low cost, but other alkalis used successfully include potassium hydroxide (15,18,19) and ammonia (20,21). Treatment of rice straw with calcium hydroxide did not improve its digestibility in one trial (22).

Table 5. Lignin content and digestibility of roughages treated with alkali for 1 hour at 60°C<sup>1</sup>

Roughage type	NaOH, %	Lignin, %	I.V.	DMD (%)
Alfalfa stem	0	14.8	47.0	
	2	14.7	41.9	
	4	15.5	50.8	
	8	15.0	54.4	
Barley straw	0	11.1	35.5	
	2	10.9	44.4	
	4	10.5	59.0	
	8	10.0	73.5	
Corn stover	0	4.8	61.3	
	2	4.9	64.6	
	4	5.0	70.4	
	8	5.1	82.2	

<sup>1</sup>Ojolade et al. (1970).

The problem of how much alkali to use has not been settled completely. In the case of sodium hydroxide some workers have used about 3 to 5% based on as-is weight of straw. Four percent seems to be a reasonable compromise for maximizing digestibility increase and minimizing unfavorable side effects. Ruminant animals can tolerate very high intakes of sodium in the diet without depressing feed intake. However, a recent lamb study at the University of New Mexico shows that a sodium intake of much higher than 2% of the diet is detrimental to weight gain and energy metabolism (23). At the 4% NaOH level, actual sodium intake is about 2.3%. Some concern has been expressed about the effect sodium ion might have on soil structure when manure from animals fed NaOH treated diets is applied as fertilizer. Consequently, as low a dietary sodium level as possible is desirable from this standpoint.

Attempts to upgrade cereal straws with sodium hydroxide have appeared in the literature since 1890. These early efforts have been summarized by Archibald (24). One process giving a highly digestible product was perfected in Germany by E. Beckmann during World War I (25). It consisted of treating straw with 8 times its weight of 1.5% NaOH solution for at least 3 hours at ambient temperature, draining off the residual liquor and washing with water until alkali free. On a weight basis 12 lbs of NaOH is required per 100 lbs of straw. Additionally, the process requires large quantities of water for washing and

leaches out 15 to 25% of the solids originally present in the straw. The process does improve digestibility; a fairly recent trial reports a 50% in vitro digestibility increase for treated oat straw over untreated material (14), although some earlier work indicates a case where a smaller digestibility increase was obtained (26). However, disposal of the strongly alkaline process water with a high BOD is simply not practical with today's environmental quality regulations. Consequently, most recent investigations on NaOH treatment of cereal wastes have aimed at modifying the original Beckmann process by reducing the NaOH and water requirements. Many investigators have suggested improved processes to upgrade straws and verified their digestibility increases by in vitro assays or by in vivo digestibility trials (11,12,17,27,28,29,30,31), but actual animal performance data are scarce. We have found that although in vitro assays are useful for ranking the results of process variable studies, and in vivo digestion trials provide an accurate assessment of true digestibility, animal performance trials are needed for accurate evaluation of the feed potential of processed materials. Some treated products have good apparent digestibilities, but do not live up to expectations in performance tests.

In 1971, a lamb performance study was reported using ground oat straw treated by the original Beckmann procedure except that some acetic acid was added to neutralize the final product (14). Treated straw level in the diet was about 65%. Table 6 indicates that this treatment of the straw gave considerable

Table 6. University of Minnesota<sup>1</sup> - Lamb trial,  
oat straw (Beckmann process)

Ration	Daily gain, g	Daily feed, Kg	Feed/gain
Untreated straw-SBM	61.5	0.87	14.6
Treated straw-SBM	177.1	1.29	7.3
Untreated straw-urea	53.1	0.82	15.3
Treated straw-urea	125.0	1.11	8.8

<sup>1</sup>Saxena et al. (1971).

improvement in growth performance. Gains with soybean meal supplement were almost 3 times better and with urea supplement about 2 times better than those with untreated straw. The authors indicate that lambs fed the treated straw--soybean meal ration gained at a rate that compared favorably with lambs fed unpelleted rations containing large amounts of concentrates. Canadian workers have also achieved fairly positive results feeding oat straw treated with 8% NaOH at a 50% moisture level for 24 hr (32). Acetic acid was added to neutralize excess alkali. Under these conditions sheep fed untreated straw were unable to maintain body weight at the levels fed (77 to 85% of the total diet). About the same time, Indian workers reported on some calf trials using finely ground wheat straw treated with NaOH at the 0, 3.3, 6.7, and 10% levels (33). Although this work was mainly a series of digestion trials, weight gain records indicated that the 3.3% NaOH treated straw out-performed all others. The 10% NaOH treated straw gave poorer gains than the untreated control in both trials, but there were no other apparent adverse effects on animal health at this high level of sodium intake.

For about the last decade, Dr. Terry Klopfenstein at the University of Nebraska has been actively investigating the upgrading of farm wastes through processing. Much of his work has had financial support from the USDA. He has looked at two different types of processes: (1) an ensiling procedure with low levels of NaOH and a 50% moisture level; and, (2) high pressure steam treatment with or without added chemicals (19). Table 7 shows the results of a lamb trial feeding an ensiled wheat straw ration (34). NaOH treated straw with soybean meal

Table 7. Performance of lambs fed wheat straw<sup>1</sup>

Ration <sup>2</sup>	Daily gain	Daily feed	Feed/gain
	lb	lb	
Straw + soybean meal	0.08	2.0	25.0
Straw + urea	-0.10	1.4	---
4% NaOH straw + soybean meal	0.35	2.7	7.6
4% NaOH straw + urea	0.18	2.2	12.4

<sup>1</sup>Klopfenstein (1973).

<sup>2</sup>Rations were 70% straw and 30% supplement.

produced very acceptable gains. The soybean supplement is the expensive component in this ration compared to urea. Subsequently, it was found that a urea-corn gluten meal supplement could be used that gave gains equal to those obtained with soybean meal but at only one half the cost. Table 8 compares

Table 8. Comparison of sodium hydroxide treated crop residues fed to lambs<sup>1,2</sup>

Roughage	Daily gain	Daily feed <sup>3</sup>	Feed/gain
	lb	lb	
Corn silage	0.29	2.0	6.8
Corn cobs	0.31	2.3	7.4
Corn stalkage	0.24	3.3 <sup>4</sup>	13.5
Milo residue <sup>5</sup>	0.37	2.8	7.4
Grass hay	0.31	2.9	9.4

<sup>1</sup>Klopfenstein (1973).

<sup>2</sup>Six lambs per treatment fed for 69 days, forage was 80% of the ration.

<sup>3</sup>Dry matter basis.

<sup>4</sup>Includes some wastage.

<sup>5</sup>Material discharged from rear of combine.

lamb performance on several ensiled NaOH (4%) treated cereal wastes (34). Treated milo residue (the material discharged from the combine) gave excellent gains with a slightly poorer feed efficiency than corn silage. A similar product called "husklage" can be obtained from corn harvesting operations. Results of 2 years of calf trials with alkali treated ensiled husklage are shown in Table 9 (35). In 1973, the alkali was 4% NaOH; in 1974, it was a

mixture of 3% NaOH and 1% Ca(OH)<sub>2</sub>. It was concluded that treated husklage has 80 to 90% of the feed value of corn silage, and that feed costs per pound of gain are cheaper from husklage than from corn silage whenever whole corn is worth more than \$2/bushel.

Table 9. Performance of calves fed treated husklage<sup>1</sup>

Roughage	Daily gain	Daily feed <sup>2</sup>	Feed/gain
	lb	lb	
<u>1973<sup>3</sup></u>			
Corn silage	1.7	13.7	8.1
Husklage	1.5	13.4	8.8
<u>1974<sup>4</sup></u>			
Corn silage	1.6	13.9	8.7
Husklage	1.4	14.3	10.4

<sup>1</sup>Klopfenstein (1975).

<sup>2</sup>Dry basis.

<sup>3</sup>12 head per treatment for 99 days. Husklage treated with 4% NaOH, moisture raised to 67%. SBM supplements.

<sup>4</sup>12 head per treatment for 105 days. Husklage treated with 3% NaOH and 1% Ca(OH)<sub>2</sub>. SBM supplements fed as 10% of corn silage rations and 20% of the husklage rations.

Using a similar ensiling technique, workers at the University of Guelph reported some bullock and heifer trials which showed that ensiled NaOH treated corn stover or barley straw could successfully replace 25% of a corn-silage, soybean diet, but that when treated materials represented 50% of the diet, performance was depressed (36).

In regard to pressure treatment, Nebraska workers have shown (Table 10) that wheat straw treated at 300 lb for 50 sec gives better lamb performance than untreated straw (19). Addition of NaOH prior to treatment gives even better performance, but it is still inferior to pelleted whole corn plant as a feed.

Table 10. Performance of lambs fed pressure treated wheat straw<sup>1,2</sup>

Treatment	Daily gain	Daily feed <sup>3</sup>	Feed/gain
	lb	lb	
Control untreated straw	0.14	1.9	19.3
Whole corn plant pellet	0.38	2.7	7.2
Treated wheat straw <sup>4</sup>	0.20	2.2	12.3
Treated wheat straw <sup>4</sup> + 3% NaOH	0.25	2.6	10.1

<sup>1</sup>Klopfenstein (1973).

<sup>2</sup>Five lambs per treatment for 56 days, straw was 70% of the ration.

<sup>3</sup>Dry matter basis.

<sup>4</sup>Treated at 300 psi for 50 seconds.

Table 11 shows the results of a lamb trial using pressure treated corn cobs (40). In contrast to wheat straw, addition of NaOH prior to pressure treatment of corn cobs gave no improvement (poorer, in fact) over pressure treatment alone. Post pressure treatment with alkali gave the best overall animal performance. The data shown in Table 12 suggest that pressure treatment of corn cobs may generate inhibitors that interfere with optimal growth of lambs (40). They show that the addition of 0.5 and 1.0% of sodium metabisulfite to corn cobs prior to pressure treatment counteracts this tendency and gives a feed-stuff as good as pelleted whole corn plant itself. The reason for the poor performance of the 1.5%  $\text{Na}_2\text{S}_2\text{O}_5$  ration is unknown.

Table 11. Lamb trial<sup>1</sup> - Corn cobs, 250 psi, 50 sec.

Treatment	Daily gains, g	Feed/gain
Untreated	87	8.5
Pressure only	186	6.2
3% NaOH, then pressure	165	6.5
Pressure, then 3% NaOH	215	6.2

<sup>1</sup>Klopfenstein *et al.* (1974).

Table 12. Lamb trial<sup>1</sup> - Corn cobs, 250 psi, 50 sec.

Treatment	Daily gains, g	Feed/gain
Pelleted whole corn plant	186	5.7
Pressure only	167	6.2
Pressure 0.5% $\text{Na}_2\text{S}_2\text{O}_3$	203	5.2
Pressure 1.0% $\text{Na}_2\text{S}_2\text{O}_3$	211	5.7
Pressure 1.5% $\text{Na}_2\text{S}_2\text{O}_3$	179	5.9

<sup>1</sup>Klopfenstein *et al.* (1974).

For about 6 years at the Western Regional Research Center, we have had a program on upgrading a variety of cellulosic wastes. Our major emphasis has been on rice straw because its disposal is a particularly important problem in California. Our efforts have involved the cooperation of several groups within our laboratory, as well as with industry members, the University of California at Davis, and Dr. Jack Simpson's Agribusiness Group of ARS-USDA. Figure 2 indicates the types of processing systems that we have looked into

1. HIGH PRESSURE STEAM TREATMENT
2. ALKALI SOAKING
3. AMBIENT TEMPERATURE AMMONIATION
4. LOW MOISTURE ALKALI COMPACTION

Fig. 2. Processes to improve digestibility of straw.

to upgrade farm wastes. At the start of our program each system was thought to offer enough unique possibilities to merit investigation on the pilot plant scale, with the goal of producing enough material for feeding trials.

The high pressure steam treatment (Fig. 3) involves treating ground straw with high pressure steam in a pressure reactor with or without added chemicals.

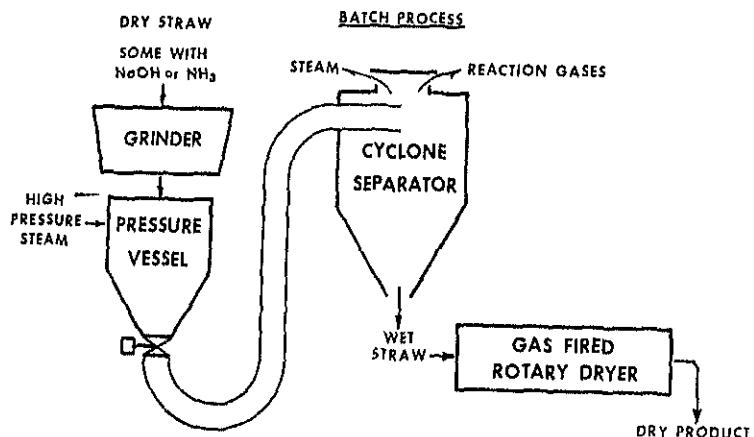


Fig. 3. High pressure steam treatment.

Our largest reactor can handle 12 cu ft of material at up to 400 psi. After the end of the reaction period (usually 10 to 90 sec) a quick release valve is opened and the product blown into a collecting cyclone. Moisture content of the product varies from 40 to 70%, requiring further drying for long term storage. Approximately 5 to 15% of dry matter is volatilized under high pressure treatment and serious air pollution results if this material is vented directly to the atmosphere. Addition of NaOH to straw prior to treatment generally gives a more digestible product, but animal performance on this material is no better than that achieved on material produced by less expensive methods (37). Overtreatment with steam is also possible, leading to poor animal performance.

The alkali soaking process represents our attempt to modify the original Beckmann process (Fig. 4). Ground straw is run through an alkali soaking

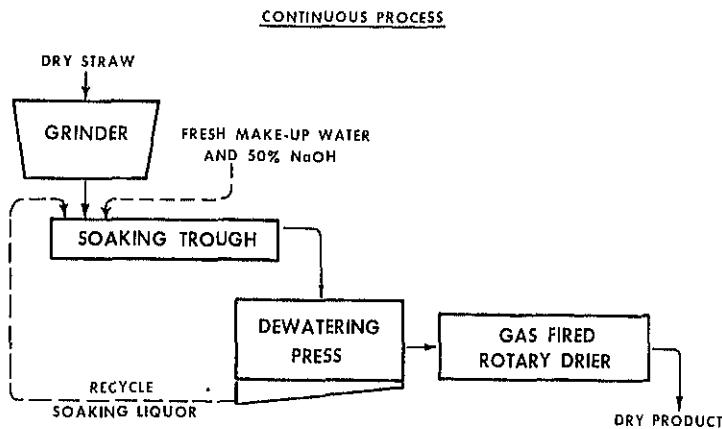


Fig. 4. Alkali soaking.

chamber at 100°C to minimize holding time. Soaked straw is pressed and dried; press waters containing excess NaOH are recycled and make-up NaOH (to maintain a 4% treatment level) and water added as needed. At equilibrium sodium ions and solubilized organic materials are discharged in the product rather than into the watershed. Treated product must be consumed immediately or dried. Animal performance on material prepared by this process is satisfactory (36).

The ambient temperature ammoniation process (Fig. 5) involves treatment of baled straw with aqueous ammonia in a sealed plastic envelope for 30 days (20). This type of process is visualized as being suitable for on-the-farm operations. Removal of residual NH<sub>3</sub> can present an air pollution problem without a mechanical collection system.

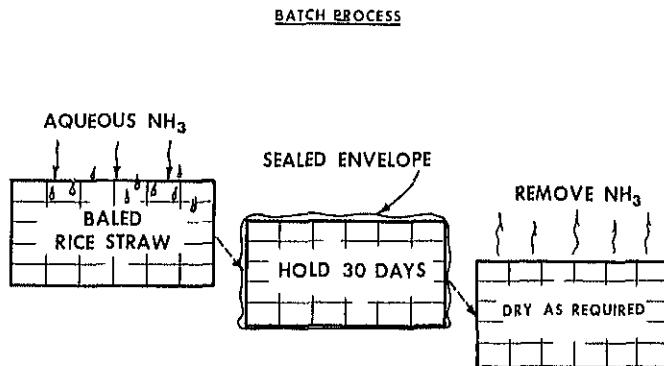


Fig. 5. Ambient temperature ammoniation.

The low moisture alkali compaction system shown in Figure 6 was first proposed by a group of Danish workers (38). Ground straw is treated with enough concentrated alkali in a mixing unit to add 4% of NaOH by weight, and then it is

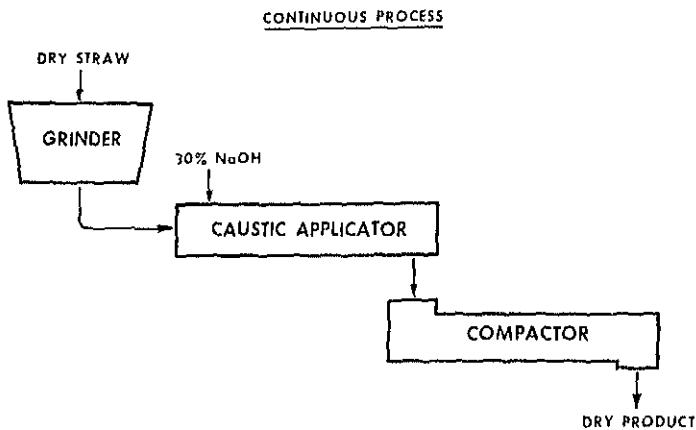


Fig. 6. Low moisture alkali compaction.

subjected to severe compaction to complete the treatment. In Denmark a cuber is used; in our work we have used either a pellet mill or a screw compactor of our own design. The primary advantages of the process are that the moisture content of the product is low enough to store safely without further drying, and that the product has a high bulk density for easy shipment. Good animal performance is obtained apparently because of the high daily intake of densified product (37).

I will conclude by presenting results of one of our many feeding trials comparing rice straw processed by several of the methods just described. Table 13 gives some lamb performance data on rations containing 72% of rice straw.

Table 13. WRRC Lamb trial, treated rice straw<sup>1</sup>

Treatment	Daily gain <sup>2</sup>	Daily feed <sup>2</sup>	Feed/gain <sup>2</sup>
	lb	lb	
Untreated control	0.20	4.09	20.4
Ambient temperature ammoniation (7%)	0.29	4.72	16.3
Alkali soaking (4% NaOH)	0.31	4.34	14.0
Low moisture alkali compaction - pelleted	0.29	4.66	16.1
Alfalfa control	0.35	3.72	10.8

<sup>1</sup>63 days, 6 animals/group, 72% ration level.

<sup>2</sup>Empty body basis.

This represents about the upper limit of straw that can be included in a diet meeting NRC requirements for lambs (39). All processes give about a 50% increase in daily gain compared to the untreated control. All give slightly (but not statistically significantly) poorer gain results than that obtained with the alfalfa control; feed efficiencies are definitely poorer.

Table 14 shows the result obtained in the same trial when treated rice straw

Table 14. WRRC Lamb trial, treated rice straw<sup>1</sup>

Treatment	Daily gain <sup>2</sup>	Daily feed <sup>2</sup>	Feed/gain <sup>2</sup>
	lb	lb	
Untreated control	0.41	4.13	10.1
Ambient temperature ammoniation (7%)	0.43	4.01	9.3
Alkali soaking (4% NaOH)	0.43	4.20	9.8
Low moisture alkali compaction - pelleted	0.50	4.57	9.1
Alfalfa control	0.35	3.72	10.8

<sup>1</sup>63 days, 6 animals/group, 36% ration level.

<sup>2</sup>Empty body basis.

made up only 36% of the ration. In this case, the control containing untreated ground straw performed just as well as the processed straws, and all straw samples at this dietary level were statistically significantly better gainwise than the alfalfa control. These results with untreated straw recall those of the English workers and others, using untreated ground straw at moderate levels (4). Results of a just completed steer feeding trial at U.C.-Davis confirm these lamb feeding data. It seems evident that to justify chemical processing to upgrade the digestibility of straws, the treated straws must be used at a high level in the diet. Whether it is economical to do this or not depends on the price and availability of alternative roughages and grains, and the comparative costs of processing. Grinding costs would be about the same for treated or untreated material, but treatment would require additional expense for chemicals and processing. Cost data are not available to resolve this problem at the present time. It seems certain, however, that, in one way or another, there will be increasing use of cereal residues in ruminant diets.

#### Literature Cited

1. U.S. Dept. Agriculture-Agricultural Statistics, 1974. U.S. Gov't Printing Office, Washington, 1974.
2. Binger, H. P., Thompson, C. R., and Kohler, G. O. Composition of dehydrated forages. USDA-ARS Technical Bulletin #1235, Washington, DC, 1961.
3. Van Soest, P. J., and Jones, L. H. P. Effect of silica in forages upon digestibility. *J. Dairy Sci.* 51: 1644, 1968.
4. a. Lamming, G. E., Swan, H., and Clarke, R. T. Studies on the nutrition of ruminants 1. Substitution of maize by milled barley straw in a beef fattening diet and its effect on performance and carcass quality. *Anim. Prod.* 8: 303, 1966.  
b. Swan, H., and Lamming, G. E. Studies on the nutrition of ruminants 2. The effect of level of crude fibre in maize based rations on the carcass composition of Friesian steers. *Anim. Prod.* 9: 203, 1967.  
c. Swan, H., and Lamming, G. E. Studies on the nutrition of ruminants 5. The effect of maize based diets containing up to 70% ground barley straw on the live-weight gain and carcass composition of yearling steers. *Anim. Prod.* 12: 63, 1970.
5. Raven, A. M., Forbes, T. J., and Irwin, J. H. D. The utilization by beef cattle of concentrate diets containing different levels of milled barley straw and of protein. *J. Agr. Sci.* 73: 355, 1969.
6. White, T. W., Reynolds, W. L., and Hembry, F. G. Level and form of rice straw in steer rations. *J. Anim. Sci.* 33: 1365, 1971.
7. Hackett, M. R., Hillers, J. K., Kromann, R. P., and Martin, E. L. Evaluation of wheat straw in feeder lamb rations. *Proc., Western Sect. Am. Soc. Anim. Sci.* 26: 143, 1975.
8. Oh, J. H., Weir, W. C., and Longhurst, W. M. Feed value for sheep of cornstalks, rice straw, and barley straw as compared with alfalfa. *J. Anim. Sci.* 32: 343, 1971.
9. Kellner, O., and Köhler, A., as cited in reference 10.
10. Woodman, H. E., and Evans, R. E. The nutritive value of fodder cellulose from wheat straw. *J. Anim. Sci.* 37: 202, 1947.
11. Chandra, S., and Jackson, M. G. A study of various chemical treatments to remove lignin from coarse roughages and increase their digestibility. *J. Agr. Sci.* 77: 11, 1971.
12. Wilson, R. K., and Pigden, W. J. Effect of a sodium hydroxide treatment on the utilization of wheat straw and poplar wood by rumen microorganisms. *Can. J. Anim. Sci.* 44: 122, 1964.

13. Tarkow, H., and Feist, W. C. A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. In Advances in Chem. Series #95. Cellulases and their applications, R. F. Gould, Editor. Am. Chem. Soc., Washington, p. 197, 1969.
14. Saxena, S. K., Otterby, D. E., Donker, J. D., and Good, A. L. Effects of feeding alkali treated oat straw supplemented with soybean meal or non-protein nitrogen on growth of lambs and on certain blood and rumen liquor parameters. J. Anim. Sci. 33: 485, 1971.
15. Unpublished work, Western Regional Research Center.
16. Stone, J. E., Scallan, A. M., Donefer, E., and Ahlgren, E. Digestibility as a simple function of a molecule of similar size to a cellulase enzyme. In Advances in Chemistry Series #95, Cellulases and their applications, R. F. Gould, Editor. Am. Chem. Soc., Washington, p. 219, 1969.
17. Ololade, B. G., Mowat, D. N., and Winch, J. E. Effect of processing methods on the in vitro digestibility of sodium hydroxide treated roughages. Can. J. Anim. Sci. 50: 657, 1970.
18. McAnally, R. A. Digestion of straw by the ruminant. Biochem. J. 36: 392, 1942.
19. Klopfenstein, T., and Koers, W. Agricultural cellulosic wastes for feed. In Symposium Processing Agricultural and Municipal Wastes. Edited by G. E. Inglett. Avi Publishing Co., Westport, Conn., p. 38, 1973.
20. Waiss, A. C., Jr., Guggolz, J., Kohler, G. O., Walker, H. G., Jr., and Garrett, W. N. Improving digestibility of straws for ruminant feed by aqueous ammonia. J. Anim. Sci. 35: 109, 1972.
21. Chomyszyn, M., Ziolecka, Z., Kuzdowicz, M., Buraczewski, S., and Kowalczyk, J. Studies in the use of ammoniated feeds in the feeding of ruminants. Roczniki Nauk Rolniczych. 84-B-1; 75, 1964.
22. Nath, K., Sahai, K., and Kehar, N. D. Effect of water washing, lime treatment and lime and calcium carbonate supplementation on the nutritive value of paddy (*Oryza sativa*) straw. J. Anim. Sci. 28: 383, 1969.
23. Jackson, H. M., Kromann, R. P., and Ray, E. E. Energy retention in lambs as influenced by various levels of sodium and potassium in the rations. J. Anim. Sci. 33: 872, 1971.
24. Archibald, J. G. The effect of sodium hydroxide on the composition, digestibility, and feeding value of grain hulls and other fibrous materials. J. Agric. Res. 27: 245, 1924.
25. Beckmann, E. The supply of carbohydrates in war: Reform of the process of rendering straw soluble. Sitz. preuss. akad., 275, 1919. Chem. Abst. 13: 2567, 1919.

26. Ferguson, W. S. The digestibility of wheat straw and wheat-straw pulp. *Biochem. J.* 36: 786, 1942.
27. Donefer, E., Adeleye, A., and Jones, T.A.O.C. Effect of urea supplementation on the nutritive value of NaOH-treated oat straw. In *Advances in Chemistry Series #95. Cellulases and their applications*. Edited by R. F. Gould. Am. Chem. Soc., Washington, p. 328, 1969.
28. Hogan, J. P., and Weston, R. H. The utilization of alkali-treated straw by sheep. *Aust. J. Agric. Res.* 22: 951, 1971.
29. Maeng, W. J., Mowat, D. N., and Bilanski, W. K. Digestibility of sodium hydroxide-treated straw fed alone or in combination with alfalfa silage. *Can. J. Anim. Sci.* 51: 743, 1971.
30. Ololade, B. G., and Mowat, D. N. Influence of whole-plant barley reconstituted with sodium hydroxide on digestibility, rumen fluid, and plasma metabolism of sheep. *J. Anim. Sci.* 40: 351, 1975.
31. Summers, C. B., and Sherrod, L. B. Sodium hydroxide treatment of different roughages. *Proceedings, Western Sect., Am. Soc. Anim. Sci.* 26: 129, 1975.
32. Javed, A. H., and Donefer, E. Alkali-treated straw rations for fattening lambs. *J. Anim. Sci.* 31: 245, 1970. (abst)
33. Singh, M., and Jackson, M. G. The effect of different levels of sodium hydroxide spray treatment of wheat straw on consumption and digestibility by cattle. *J. Agr. Sci.* 77: 5, 1971.
34. Klopfenstein, T. Treatments to increase the value of crop residues for beef cattle and lambs. *Proceedings of the Distillers Feed Research Council* 28: 24, 1973.
35. Klopfenstein, T. Evaluation of chemically treated crop residues. *Proceedings-Eighth Research-Industry Conf. American Forage and Grassland Council*, Lexington, Ky, p. 37, 1975.
36. Mowat, D. N. NaOH-stover or straw silage in growing rations. *J. Anim. Sci.* 33: 1155, 1971. (abst)
37. Garrett, W. N., Walker, H. G., Jr., Kohler, G. O., Waiss, A. C., Jr., Graham, R. P., East, N. E., and Hart, M. R. Nutritive value of NaOH and NH<sub>3</sub> treated rice straw. *Proceedings, Western Sect. Am. Soc. Anim. Sci.* 25: 317, 1974.
38. a. Rexen, R. P. Forøgeise af halms forøjelighed ved kenisk behandling. (Increasing the digestibility of straw by chemical treatment) *Usekrift for Agronomer og hortonomer* 18: 364, 1972.  
b. Rexen, F., and Moller, M. Use of chemical methods to improve the nutritional value of straw crops. *Feedstuffs* 46(8): 46, 1974.

39. National Academy of Sciences-National Research Council. Nutrient requirements of sheep. Revised edition, Washington, DC, 1968.
40. Klopfenstein, T., Graham, R. P., Walker, H. G., Jr., and Kohler, G. O. Chemicals with pressure-treated cobs. J. Anim. Sci. 39: 243 (1974).

CEREAL STARCH AND FLOUR PRODUCTS AS SUBSTITUTES AND  
EXTENDERS FOR PETROLEUM-BASED POLYMERS AND PLASTICS

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Recent, as well as projected, price increases and shortages of petroleum and natural gas have created much interest in using annually renewable raw materials to develop substitutes and extenders for synthetic polymers and plastics. Cereal starches and flours are attractive candidates because of their abundance, low cost, polymeric nature, and ease of conversion into useful intermediates. For applications where biodegradability is desired, cereal products provide an added attraction.

Currently, 22 billion pounds of plastics and about 5 billion pounds of synthetic elastomers (rubber polymers) are produced annually in the U.S. from petrochemicals. In addition, several hundred million pounds of petroleum-based synthetic polymers are produced for use in adhesive, thickening, flocculating, and coating applications. Thus, there is ample incentive to seek means for sparing the use of petrochemicals in the production of synthetic polymers and plastics. During the past several years, a number of cereal-based products and processes have been developed that have good potential for satisfying some of the pressing needs.

Polyurethane Foams

One type of plastic that affords an excellent opportunity for incorporation of starch-derived materials is rigid polyurethane foam. This relatively new plastic is experiencing a remarkable growth rate, particularly in construction, and is projected to reach the billion pound per year level by 1980. Rigid urethane foams gained rapid acceptance because they have about twice the insulating value of any other commercially available material, are easy to apply, and give a high strength-to-weight ratio. Such foams in houses and other buildings conserve heating and cooling energy and reduce construction costs.

Urethane foams are made by crosslinking propoxylated polyols with di- or poly-isocyanates in the presence of a blowing agent (Freon), a surfactant, and a catalyst. Foaming occurs due to vaporization of the Freon by the heat of reaction. Isocyanates react with hydroxyl groups on polyether polyols to form a three-dimensional network polymer in which the reactants are tied together by urethane linkages ( $-\text{NHCO}_2-$ ) formed during the reaction. Polyols that have been used most extensively in urethane foam are pentaerythritol (a petroleum-derived polyol) and sucrose. Recent gyrations in the price and availability of sucrose virtually eliminated its use and dimmed its future in urethane foams.

In view of increased costs and shortages of pentaerythritol and sucrose, the urethane foam industry will most likely take a second look at starch-derived polyols we developed several years ago (12,16). The method for making these polyols is depicted in figure 1. It involves

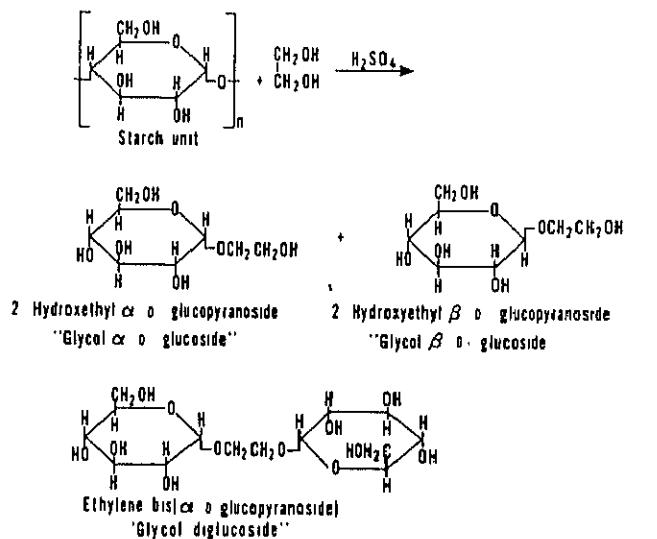


Figure 1.--Preparation of polyols from starch.

heating starch in the presence of excess ethylene glycol and an acid catalyst. When the reaction is completed, the mixture is made alkaline and excess ethylene glycol is removed under vacuum. The product is a mixture comprised mostly of monoglucosides and need not be separated for urethane foam production. The crude polyol mixture is treated with about 6 moles of propylene oxide per mole of polyol to give polyether polyols (fig. 2). These are then converted to rigid urethane foam by

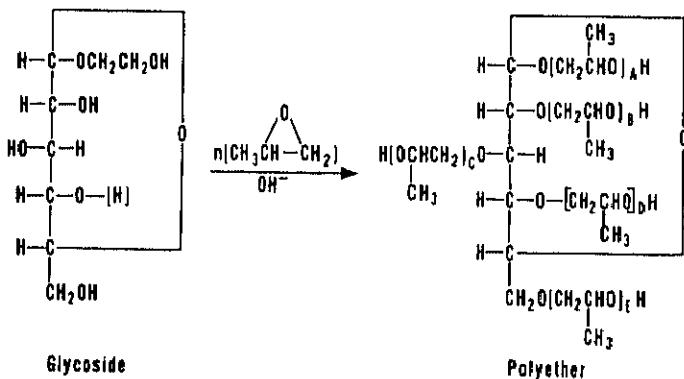


Figure 2.--Etherification of polyols.

treatment with a di- or poly-isocyanate in the presence of a blowing agent, a surfactant, and a catalyst. In exhaustive evaluations, rigid urethane foams made from starch-derived polyols met or exceeded all specifications. Moreover, the cost to make starch-derived polyols has been estimated from scaled-up operations (12) and is well below that of pentaerythritol. In addition, a continuous process for making polyol-type products from starch has been disclosed, which may lead to some reduction in cost (10).

Although urethane foam without added flame retardants is used as an insulator in many places, such as refrigerator walls, where fire is not a likely hazard, flame retardancy must be imparted to foams used in buildings. This characteristic has been accomplished by adding halogen, phosphorus, and antimony compounds either alone, in selected combinations, or by chemically bonding these elements to one reactant or both. By these means, flame retardancy has been good. However, improvement is still needed because present flame retardant urethane foams must be faced or sandwiched between fireproof material, such as mineral board, to meet housing codes.

Flame retardant technology has been advanced through the promising development of halogenated glucose-based polyether polyols (figs. 3 and 4). Glucose, obtained from starch by hydrolysis, is reacted with allyl alcohol to form allyl glucoside which, in turn, is propoxylated and brominated (15). These halogenated polyols impart greater flame resistance to urethane foams than other polyols having equal halogen content because of the strategic location of the halogens and the cyclic structure of the polyol.

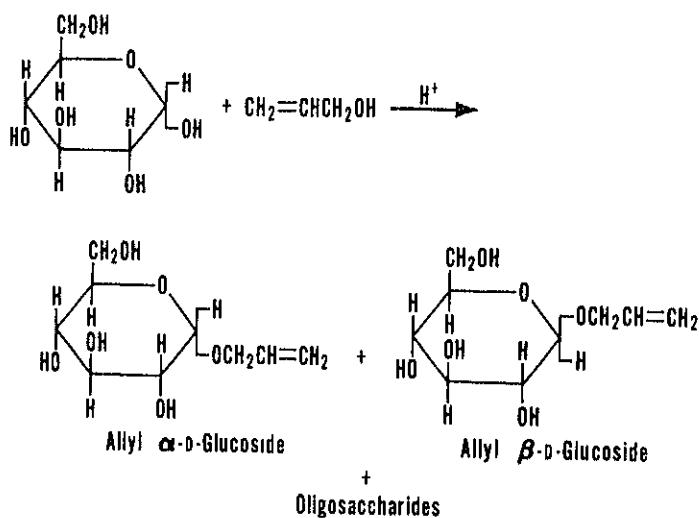


Figure 3.--Preparation of allyl glucosides.

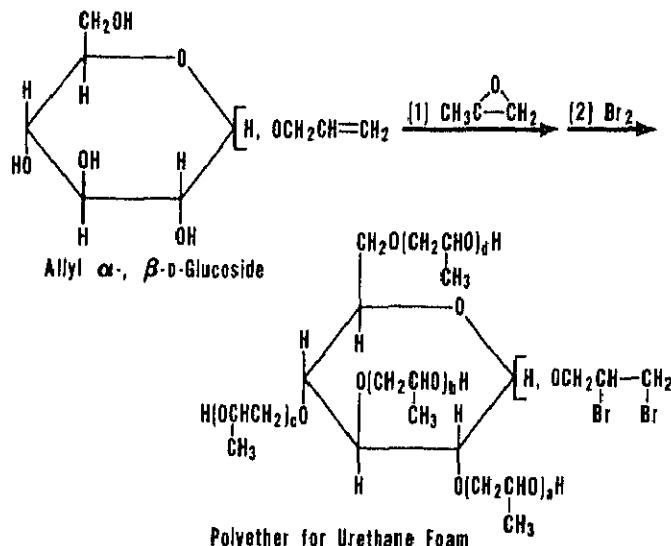


Figure 4.--Propoxylation and bromination of allyl glucosides.

### Alkyd Resins

Approximately 600 million pounds of alkyd resins are produced annually in the United States. Their principal use is as protective coatings (paints) on automobiles, metal and wood furniture, refrigerators, stoves, and walls. Alkyds are made by reacting a polyol with a dibasic acid anhydride and one or more other ingredients (usually an unsaturated triglyceride or an unsaturated fatty acid) as shown in figure 5. The product is mainly a mixture of polyesters formed by esterification and ester interchange, but some etherification also occurs, especially at temperatures above 210° C. Through variations in the nature and proportions of polyol, anhydride, and other components, the range of products is almost limitless. Again, the principal polyols used are petrochemically derived. However, polyols derived from starch as previously described are capable of replacing up to 85% of the synthetic polyols without any loss of properties (13).

### Biodegradable Films and Plastics

To alleviate disposal problems, films and rigid plastics that will biodegrade are being sought for many applications. One multimillion pound-per-year use of plastic film, where a biodegradable film is sorely needed, is the mulching of horticultural crops. Currently, thousands of acres of such crops are mulched with polyethylene film. Application of the mulch film usually involves covering a prepared row of soil with a strip of plastic film of appropriate width and then covering the edges

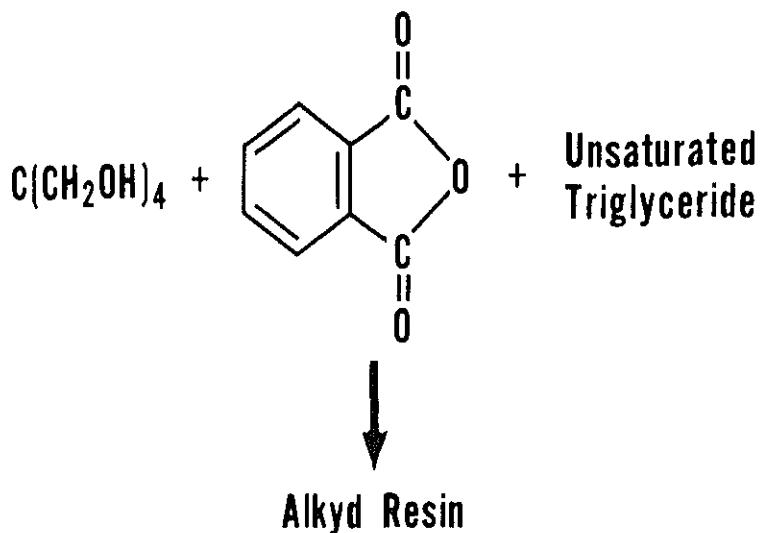


Figure 5.--Formation of alkyd resins.

of the film strip with soil to hold it down. Seedlings or seeds are then planted through holes punched in the film. Plastic mulch often doubles the gross income from crops by reducing nutrient leaching, preventing weed growth, and controlling soil temperature and moisture. The trouble with polyethylene film is that it does not degrade and must be removed between growing seasons at a cost of \$40.00 to \$100.00 per acre. Disposal of the film is also a problem because burning is prohibited and landfill sites are becoming scarce.

Recent studies on the incorporation of cereal starches into plastic films indicate that prospects for producing a satisfactory biodegradable film are good (8,14,19). Starch-polyvinyl alcohol (PVA) films containing up to 64% starch have been made that appear to have the required functional properties. Thin coatings of Saran or polyvinyl chloride were applied to these films to reduce their sensitivity to moisture and to control their rate of degradation. Starch-PVA films are produced by casting aqueous dispersions containing 12 to 15% solids on a continuous stainless-steel belt and then drying.

This process, known as band casting, is used commercially for making some types of films but is more expensive than hot-melt extrusion. Consequently, starch-PVA films were judged to be a bit too costly at the time they were first disclosed (about 2 years ago) to compete with polyethylene as a mulch film even though credit was given for the cost of removing the polyethylene mulch. If the cost of polyethylene continues

to rise as predicted, the band-cast starch-PVA films may become competitive with polyethylene films. Other film-forming processes, including hot-melt extrusion of starch-PVA composition, are being investigated and may lead to the development of lower cost films.

The current starch-PVA film technology has paid off as it is being used commercially with minor modifications to produce water-soluble laundry bags (11). The modification involves the use of a slightly derivatized starch in place of pearl starch to impart solubility in water. The bags are used in hospitals and other institutions to keep the laundry staff from coming in contact with soiled or contaminated clothing and bedding collected in them. The unopened bags are thrown into the wash where the bag readily dissolves.

Starch-polyvinyl chloride (PVC) films and plastics that are biodegradable have also been developed (19). Three different methods for incorporating starch in PVC have proved successful. In the first, starch xanthate solution is added to a PVC latex (an aqueous emulsion of PVC); then  $\text{NaNO}_2$  and an alum are introduced to convert (crosslink) oxidatively the xanthate to the insoluble xanthide, which coprecipitates with the PVC. The coprecipitate is collected on a filter, dried, and ground to a fine powder for subsequent processing into films or solid plastic articles. Type reactions for xanthation and oxidative coupling are shown in figure 6. With starch, only low levels of xanthation are needed corresponding to degrees of substitution in the 0.08 to 0.10 range.

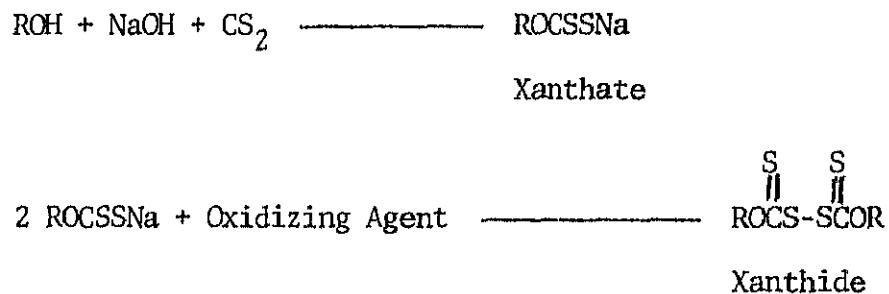


Figure 6.--Xanthate and xanthide formation.

In the second method, whole starch is gelatinized by heating in water before mixing with the PVC latex; then water is removed from the mixture in a forced-air oven and the product ground.

In the third method, whole starch is dry blended with solid PVC and dioctyl phthalate (a plasticizer) on a rubber mill. The mixture is then sheeted into a film by passage through the rolls or is molded under pressure into rigid plastic articles in a heated mold. The same procedures are used to incorporate plasticizer and to make films and plastics from the dry starch-PVC powders obtained in methods 1 and 2.

Starch-PVC films containing from 25 to 44% starch in Weatherometer tests lasted from 40 to 900 hours, depending on the level of starch and method of incorporation. In outdoor exposure to soil, a few samples deteriorated in 30 days, whereas others lasted for more than 120 days. It appears that starch-PVC films can meet cost and performance requirements for agricultural mulching. However, further processing studies and testing are needed to reach a definite conclusion.

Evaluation of starch-PVC plastics prepared by the methods described shows that with all three, up to 40% starch can be incorporated with little loss in tensile strength. However, plastics made by the dry-blend method are fairly opaque, whereas those made by the other two methods have good clarity. All plastics that contain 12% or more starch are biodegradable because they support heavy mold growth when inoculated with soil microorganisms.

Although the preparation of biodegradable starch-filled polyethylene films has been announced (8), enough data on strength properties and rate of degradation have not been provided to assess their potential utility for various applications, including crop mulching.

In all the studies reported here on PVA and PVC, starch has been the extender or filler used. However, our latest research at the Northern Laboratory with low-protein wheat flours indicates that they can usually be substituted for starch without any significant change in overall properties of the end products.

#### Rubber

Current domestic consumption of carbon blacks as reinforcing agents for rubber amounts to about 3.5 billion pounds per year. These carbon blacks are made from petroleum fractions by inefficient partial combustion processes. Faced with the wasteful nature of the processes and projected price increases and shortages of petroleum, the rubber industry perceived some time ago the need for carbon black substitutes. A potentially useful substitute for low- and medium-reinforcing grades of carbon black has been developed from cereal starches and flours (3,4). These substitutes are cereal xanthides which are prepared at low cost by the type reactions presented in figure 6.

Cereal xanthides are incorporated into rubber by addition of the xanthate to latex (an aqueous emulsion of rubber polymer) followed by addition of sodium nitrite and acid (fig. 7). In the process, the

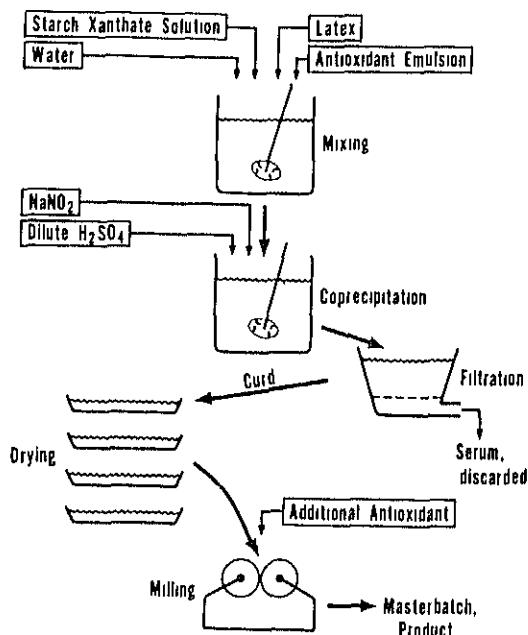


Figure 7.--Incorporation of starch xanthide in rubber.

xanthate is adsorbed on the rubber particles and insolubilized by oxidative crosslinking through the action of nitrous acid generated from the sodium nitrite. Simultaneous destabilization of the latex occurs to give a precipitate of intimately mixed rubber polymer and cereal xanthide. The supernate or serum is removed from the precipitated xanthide-rubber crumbs on a wire or cloth screen, and the crumbs are dried in an oven or by passage through a heated extruder. The dried product when processed in the usual manner by addition of curing agents, antioxidants, and other ingredients, followed by vulcanization yields reinforced rubber. The principal types of synthetic rubbers, as well as natural rubber, when reinforced with cereal xanthides have the required properties for many applications. In fact, 50-50 blends of cereal xanthide- and carbon black-reinforced rubbers in limited evaluations as tread rubber for tires have shown good wear properties. In applications where low sensitivity to moisture is required, this property is readily imparted to xanthide-reinforced rubbers by incorporating small amounts of resorcinol-formaldehyde resin or amino-silanes (3). Cost analyses indicate that the method for incorporating cereal xanthides in rubber is economically sound. Moreover, the process is compatible with the usual manufacturing operations in the rubber industry.

The development of low-cost powdered rubbers that require only a small amount of partitioning agent or coating on the rubber particles to prevent caking has been a long-sought industrial goal. Such powdered rubbers are desired because they can be formulated, mixed, and processed with much less expenditure of energy than is needed when conventional slab or baled rubber is used. Cereal xanthides are effective coating or encapsulating agents for latex particles in making powdered rubbers. The process for making powdered rubber is the same as that shown in figure 7 with the following exceptions: The extent of xanthation (degree of substitution) of starch is raised from 0.08 to around 0.25 and rates of addition of reactants and intensity of mixing are altered (1,2). With these processing changes, the coated rubber precipitates in a finely divided state, instead of in large curds, and does not cake on drying. These powdered rubbers are easily mixed with reinforcing carbon black, curing agents, and other ingredients, with the expenditure of only about one-half the energy required when conventional slab rubber is used. While the small amount of starch xanthide present in powdered rubber replaces only an equivalent amount of petroleum-derived carbon black, the energy savings in processing are equivalent to a significantly large amount of either fuel oil or natural gas.

### Graft Polymers

Graft polymerization of starch with synthetic vinyl-type monomers has produced numerous new polymers that have potential utility in many applications now served wholly by synthetic polymers derived from petrochemicals (6,7). Grafting can be conducted in various ways (fig. 8).

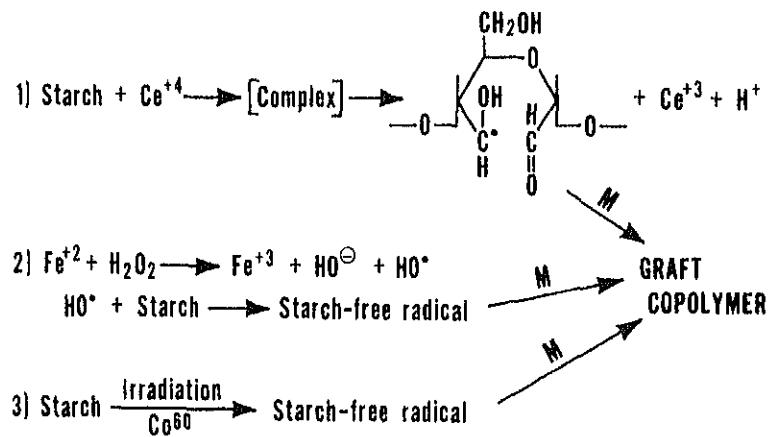
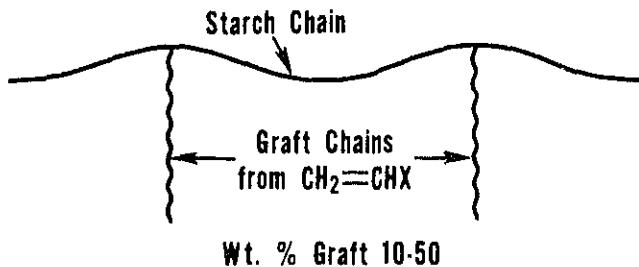


Figure 8.--Grafting synthetic polymers to starch; M = synthetic monomer(s).

It involves the generation of free radical sites on the starch by use of chemical initiators or high energy radiation. Added vinyl-type monomers (M) are induced to polymerize (form graft chains) at the free radical sites on the starch to give part starch and part synthetic polymers firmly bound together by strong chemical bonds. These graft polymers, as opposed to physical mixtures of starch and synthetic polymer, cannot be separated into their component parts by ordinary means. Moreover, many other properties of the graft polymers are different and usually superior to those of physical mixtures. An almost unlimited number of starch graft polymers can be made by varying the type of starch, the type of vinyl monomer or comonomers, the extent of grafting, and the length and number of graft chains attached to the starch. This range of products and the effect of monomer on solubility characteristics of starch graft polymers are illustrated in figure 9. When X on the monomer or comonomer is a highly polar group, the products are either water soluble or highly dispersible in water; whereas, water-insoluble products result when X is nonpolar.



When  $X = -CO_2H, -CONH_2, -CO_2(CH_2)_n-NR_3^+Cl^-$   
products are  $H_2O$  soluble and useful as thickeners,  
absorbents, sizes, adhesives, and flocculants.

When  $X = -CN, -CO_2R, -C_6H_5$   
products are  $H_2O$  insoluble and potentially useful  
as resins and plastics.

Figure 9.--Structure and properties of starch graft polymers.

Some water-soluble graft polymers of starch made to date are excellent thickening agents (7) for aqueous systems, whereas others are good flocculating agents for suspended solids in wastewaters (5). A number of cationic and anionic graft polymers exhibit potential utility in the beneficiation of ores and minerals (5,7), and some of the cationic graft polymers are excellent retention aids for pigments applied at the wet end in papermaking (9).

The graft copolymer that has received the most acclaim to date is one prepared by saponification (fig. 10) of starch-polyacrylonitrile graft polymer containing about 50 weight percent grafted polyacrylonitrile (17,18). Even though this polymer when dried on the neutral-to-alkaline side is water insoluble, yet it absorbs up to 1000 times its weight of

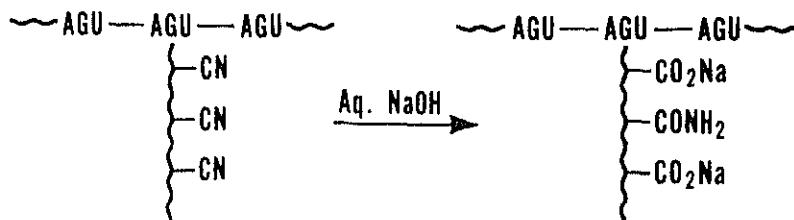


Figure 10.--Saponification of starch-polyacrylonitrile graft polymer.

distilled water. Although the presence of salts reduces its absorbency considerably, the product will absorb about 80 times its weight of simulated urine. The unusual absorptive properties of this polymer have made it a prime candidate for use in disposable diapers and numerous other applications, including coatings for seeds and roots of seedlings and soil treatment to conserve moisture. This graft polymer in worldwide competition won an IR-100 award sponsored by "Industrial Research" magazine as being one of the hundred most novel and useful products introduced in 1975. This product is now being manufactured on a limited scale, and large-scale commercialization is planned. In addition, three or four other graft polymers of starch developed at the Northern Laboratory are being intensively investigated for purposes of commercialization.

In summary, a number of products and processes have been developed that can contribute significantly to the use of cereal products as reactive components or extenders in synthetic plastics and polymers. I feel that progress made so far is only the beginning of what may be accomplished with further research and development.

### Literature Cited

- (1) Abbott, T. P., James, C., Doane, W. M., and Russell, C. R. Elastomers encased in a little starch...could put starch in powdered rubber mart. *Rubber World* 169(6):40-43. 1974.
- (2) Abbott, T. P., James, C., Doane, W. M., and Russell, C. R. Injection molding of conventional formulations based on starch-encased powdered rubber. *J. Elast. Plast.* 7(2):114-132. 1975.
- (3) Buchanan, R. A., Doane, W. M., Russell, C. R., and Kwolek, W. F. Starch xanthide reinforced styrene-butadiene rubber: Compounding to reduce water sensitivity. *J. Elast. Plast.* 7(2):95-113. 1975.
- (4) Buchanan, R. A., Kwolek, W. F., Katz, H. C., and Russell, C. R. Starch in rubber. Influence of starch type and concomitant variables in reinforcement of styrene-butadiene rubbers. *Staerke* 23(10): 350-359. 1971.
- (5) Burr, R. C., Fanta, G. F., Doane, W. M., and Russell, C. R. Starch graft copolymers for water treatment. *Staerke* 27(5):155-159. 1975.
- (6) Fanta, G. F. Synthesis of graft and block copolymers of starch. In "Block and graft copolymerization," ed. R. J. Ceresa, vol. 1, chap. 1, pp. 1-27. New York. 1973.
- (7) Fanta, G. F. Properties and applications of graft and block copolymers of starch. In "Block and graft copolymerization," ed. R. J. Ceresa, vol. 1, chap. 2, pp. 29-45. New York. 1973.
- (8) Griffin, G. J. L. Biodegradable fillers in thermoplastics. *Am. Chem. Soc., Div. Org. Coat. Plast. Chem.*, Pap. 33(2):88. 1973.
- (9) Heath, H. D., Hofreiter, B. T., Ernst, A. J., Doane, W. M., Hamerstrand, G. E., and Schulte, M. I. Cationic and nonionic starch graft polymers for filler retention. *Staerke* 27(3):76-82. 1975.
- (10) Hughes, J. F. Polymeric starch composition. U.S. Pat. 3,778,392. 1973.
- (11) Kirby, K. W., and Willey, B. L. Starch derivatives compatible with poly(vinyl alcohol). *Abstr. Pap.*, 170th national meeting, Am. Chem. Soc., Carbohydr. Div., Abstr. 45. 1975.

(12) Leitheiser, R. H., Impola, C. N., Reid, R. J., and Otey, F. H. Starch-derived glycol glycoside polyethers for urethane foams. Process scale-up, performance in foams, and cost estimates. *Ind. Eng. Chem. Prod. Res. Develop.* 5(3):276-282. 1966.

(13) McKillip, W. J., Kellen, J. N., Impola, C. N., Buckney, R. W., and Otey, F. H. Glycol glycosides in alkyds. *J. Paint Technol.* 42(544):312-319. 1970.

(14) Otey, F. H., Mark, A. M., Mehltretter, C. L., and Russell, C. R. Starch-based film for degradable agricultural mulch. *Ind. Eng. Chem. Prod. Res. Develop.* 13(1):90-92. 1974.

(15) Otey, F. H., Westhoff, R. P., and Mehltretter, C. L. Flame-retardant rigid polyurethane foam from allyl glucosides. *J. Cell Plast.* 8(3):156-159. 1972.

(16) Otey, F. H., Zagoren, B. L., and Mehltretter, C. L. Rigid urethane foams from glycoside polyethers. *Ind. Eng. Chem. Prod. Res. Develop.* 2(4):256-259. 1963.

(17) Weaver, M. O., Bagley, E. B., Fanta, G. F., and Doane, W. M. Gel sheets produced by hydration of films from the potassium salt of hydrolyzed starch-polyacrylonitrile graft copolymer. *Appl. Polym. Sci., Symp.* No. 25:97-102. 1974.

(18) Weaver, M. O., Fanta, G. F., and Doane, W. M. Highly absorbent starch-based polymer. *Proc. Tech. Symp. Nonwoven Product Technol. Int. Nonwovens Disposables Assoc.*, Washington, D.C., March 5-6, 1974, pp. 169-177. 1974.

(19) Westhoff, R. P., Otey, F. H., Mehltretter, C. L., and Russell, C. R. Starch-filled polyvinyl chloride plastics--preparation and evaluation. *Ind. Eng. Chem. Prod. Res. Develop.* 13(2):123-125. 1974.

NUTRIENT CONTENT OF WHEAT AND WHEAT PRODUCTS:  
Present Knowledge and Problems to be Solved

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This paper outlines our present knowledge of nutrient composition of wheat and its milled, processed, and baked products. This review was made to aid in the compilation and evaluation of data for the Nutrient Data Bank (NDB) and for the revision of Agriculture Handbook 8, "Composition of Foods--Raw, Processed, Prepared" (1). Handbook 8, which is now being revised, will be produced as rapidly as possible in sections according to food groups.

Since wheat and wheat products comprise over 80 percent of the grain products in U.S. diets, it is imperative that accurate values for nutrient content be available for these foods.

PRESENT KNOWLEDGE OF FACTORS INFLUENCING NUTRIENT CONTENT

WHOLE GRAIN: Research in many areas of the world has shown that a variety of factors influence nutrient content of the grain (2, 3). These factors include type of wheat, cultivar, crop year, location of growth, soil, rainfall, seed maturity, fertilization, irrigation, date of planting, and seeding rate. Of course, the grain as such is seldom consumed as food, but rather as the milled, processed, or baked product. In past years, cereal chemists and wheat breeders have been primarily interested in total protein and lysine content of the grain.

The influence of type of wheat on protein content and amino acid pattern has been investigated by a number of cereal scientists (4, 5, 6, 7) over a period of years and found, in most cases, to be significant. Hard, soft, and durum wheats differ in amounts and types of carbohydrate and in lipid components as well as in many of the vitamins and minerals. In a comparison of carbohydrate content of types of wheat, durum was highest in fiber, 2.5 percent compared with 2.2 and 2.3 percent for the other two types; soft wheat, lowest in cellulose was 2.1 compared with 2.4 percent for the others (8). Soft wheat contained 59 percent starch compared with 56 and 55 percent, respectively, for hard and durum wheats (8). Highest of the types of wheat in lipid content were the durums (Figure 1) (9). Hard and soft wheats were shown to be higher than durum wheats in alpha tocopherol (Figure 2) (10). Durum wheats, on the other hand, were found by Hepburn (11) to be 50 percent higher in total niacin than either soft or hard wheats. Hepburn and Tulloss (12) also demonstrated that durum wheats were slightly higher than hard or soft wheats in phosphorus (Figure 3). Soft wheats were low in magnesium compared with durum or hard wheats (Figure 4). Content of the vitamins thiamin and riboflavin (3, 13), and the minerals zinc, molybdenum (14), copper, and manganese (15), have been shown to be influenced by both type and cultivar of wheat.

In research on cultivars of high protein winter wheats, Mattern et al (5)

found that crosses of Atlas 66 with Comanche or Atlas 66 with Wichita were higher in protein than either Comanche or Wichita alone. A significant positive correlation was also reported by Deosthale et al between protein and lysine content of the wheat grain (16). Shoup et al (17) found that the proteins of hard red spring wheats contained less of the amino acids, lysine, arginine, and methionine and more cystine than did hard red winter wheats.

Other factors--crop year, location, soil, rainfall, seed maturity, fertilization, irrigation--also affected nutrient content of wheat grain. As demonstrated in a comparison of cultivars of durum wheat, crop year influenced protein content (18). In that research, the cultivars--Lakota, Mindum, and Sentry--were consistently lower in protein content in one crop year, 1962, than in the previous year, 1961. The limiting amino acid, lysine, in wheat protein was shown to be unaffected by factors such as soil, rainfall, or fertilizer (19).

In a comparison of three cultivars of Kansas-grown wheat from 13 locations, Schrenk and King (20) found that location was much more important than cultivar. Ash and mineral content varied appreciably within the state, and the areas producing wheats of high mineral content also produced wheats of high protein content (20). In grain grown in southwestern Kansas, in the three years, 1943-1945, manganese content of wheat was highest in 1943 (19).

A large amount of iron in the soil was reported by King and Perkins (21) to reduce the phosphorus content of wheat. Kuhn and Schaumloffel (22) demonstrated that increased copper in the soil produced an increase in zinc content of wheat. Soil and rainfall are factors that interact with location of growth. Optimal soil moisture (19) during the proper stage of plant growth produced a grain high in manganese.

Shellenberger and Schrenk, in a comparison of nutrient content of Peruvian and Kansas-grown wheats in 1948 (23), found the former to be lower in phosphorus, magnesium, manganese, copper, and potassium. They attributed the low values in Peruvian wheats to the depleted soils in which they were grown.

In research at North Dakota State University on changes due to maturation of wheat, two cultivars each of hard red spring and durum wheats showed a three-fold increase in average lipid content of the kernel with a steady decline in ash content (24). As maturation of the grain progressed, these authors (25) also reported that triglycerides increased uniformly, whereas monoglycerides decreased and diglycerides remained essentially constant. At Kansas State University, Abernethy, Paulsen, and Ellis (26), found that zinc in the winter wheat cultivar, Parker, increased steadily during maturation of the grain while phosphorus was translocated from the vegetation into phytic acid in the grain. As wheat matures, the carbohydrate composition changes by increasing in pentosan and starch, and the starch components amylose and amylopectin (27). Cerning and Guilbot (28), on the other hand, found somewhat different results with two cultivars in France. Mono-, di-, and oligo-saccharides decreased with maturation in both the Cappelle and the Joss cultivars.

Increases in nitrogen fertilization cause increases in protein content of wheat grain (29, 30, 31) but have no influence on content of the minerals iron, zinc, manganese, and copper (32). Fine (33), however, reported that nitrogen fertilization had a beneficial effect on magnesium content of grain. Spring application of fertilizer (34) caused a higher protein content in two cultivars of soft winter wheat, Blueboy and Redcoat, than did fall application of fertilizer. In studies with dwarf wheats at the Indian Agricultural Institute, scientists found increases in protein content with increases in soil fertilizer levels and a concomitant increase in the amino acids glutamic acid, phenylalanine, proline, and leucine (35). In Iran, scientists demonstrated that percent of lysine in the protein was inversely related to nitrogen levels and protein content of the grain (36). Gately reported in the Irish Journal of Agricultural Research in 1971 (37) on response to nitrogen fertilization of the spring wheat cultivar Quern, grown in 2 crop years, 1967 and 1969. The protein content of the grain was much higher in 1967 than in 1969. In 1967, the wheat crop followed a grass crop, and in 1969 it followed crops of potatoes and rutabagas.

Irrigation effects, date of planting, and seeding rate influenced content of iron, zinc, manganese, and copper which proved to be positively correlated with protein value (32). In 1972, Dubetz (29) found that wheats of high protein content could be grown on irrigated land. Greaves and Heist, in Utah studies (38), demonstrated that increased irrigation was associated with mineral composition of wheat, causing increases in calcium and magnesium content. In other research, iron, chlorine, sulfur (39), and phosphorus (40) increased with irrigation.

In a study on effects of fumigation and storage of wheat the vitamin B<sub>6</sub> values of the grain were unchanged (41).

MILLED FLOUR: It is well known that the process of milling wheat into white flour causes a drastic loss of nutrient content. Factors affecting nutrients in flour include extraction rate and type and cultivar of wheat. More correctly, the wheat grain, when milled, loses its vitamins and minerals mostly to the bran, shorts, germ, and red dog--and the resulting highly refined flour is relatively low in nutrients. Enrichment of flour with the B-vitamins thiamin, riboflavin, and niacin, and the mineral iron offsets only a few of these losses. The standards for enrichment of the B-vitamins per 100 grams of flour were increased, effective July 1, 1975, to 0.64 mg thiamin, 0.40 mg riboflavin, and 5.3 mg niacin. The proposal to increase iron to 8.8 mg has been stayed.

Because significant levels of protein are in the bran and germ, the milled flour contains less protein than the original wheat grain. Protein content is significantly and positively correlated with extraction rate (42). It has also been related to specific cultivars. Patterns of amino acids of different types of wheat flour (43) and farina (44) are quite similar. The lysine content of flour has been found to be approximately 30 percent lower on a gram per 100 grams of protein basis than that originally present in the wheat (45).

Hepburn and Tulloss (12) also found much lower values for some nutrients

in flour than in their respective wheat grains. Iron, calcium, phosphorus, and potassium were much lower in flour than in wheat grain (Table 1). In a comparison of mill fractions, Waggle et al (46) reported that flour was lowest in calcium, phosphorus, potassium, sodium, and magnesium. As is well known, various high-protein, high-vitamin wheat concentrates of different types have been developed from mill fractions for food use in the past few years.

Except for the loss of calcium, which was approximately the same for all wheats, the nutrient losses in milling semolina from durum wheat were much less than in milling flour from soft or hard wheats. In 1964, scientists from the American Institute of Baking reported on mineral content of wheat flour compared with that of the grain (47). Calcium retention was approximately 40 percent, while the retention of minerals, magnesium, manganese, phosphorus, potassium, zinc, iron, and copper ranged from 20 to 32 percent. Ferretti and Levander (48) reported that farina and wheat flour contained 71 and 86 percent, respectively, of the selenium from the original wheat. Mineral element content of whole wheat flours, of course, is similar to that of the wheats from which they were milled.

Hepburn (49) found that thiamin and riboflavin were lowest in cake flours and highest in cut-off flours--reflecting differences in extraction rate. Riboflavin was influenced far less than was thiamin. The differences in effects of milling on these two vitamins have been related to their relative distribution within the wheat kernel (50). Schultz et al in 1939 (51) reported that 82 percent of the thiamin in wheat is in feeds. They also reported that patent flour contains only 7 percent of the thiamin of wheat. Waggle et al (46), in a study of hard winter, hard spring, and soft winter wheats in different geographical areas, reported that millfeeds contained more of the B-vitamins and alpha tocopherol than did flour. Folacin content was consistently higher in whole wheat flour than in clear flour and lowest in patent flour (52).

Semolina contained higher proportions of the total niacin of durum wheats than flours milled from the respective soft and hard wheats (11). That is, semolina contained 35 percent of the niacin of the grain, whereas bread flours and cake flours contained 28 and 14 percent of the niacin of their respective grains (53). For total vitamin B<sub>6</sub>, the milled semolina, bread flour, and cake flour contained 28, 15, and 10 percent, respectively, of that in durum, hard, and soft wheat grains. For tocopherol content of semolina, bread flour, and cake flour, the milled products contained 43, 11, and 6 percent of that in their respective grains (53).

Zook et al (54) reported drastic reductions in the mineral element contents of manganese, nickel, copper, zinc, magnesium, tin, and chromium when hard, soft, and durum wheats were milled into their respective flours and semolina. Differences between wheat and the flour or semolina were usually not as great for the durum wheats as for the hard or soft wheats (Table 2). However, values for nickel in flour or in semolina were about the same for all wheats.

Bakers' flour contains 2 to 3 percent total pentosans (55)--complex

carbohydrates. Pentose-rich fractions have not been found to be significantly correlated with normal variation in milling procedures (42). In a comparison of carbohydrate composition of flours from hard, soft, and durum wheats, semolina contained the most reducing sugar and the least starch (8). Hard wheat flour was low in reducing sugar and starch, and cake and cracker flour contained the most starch (8). Al-Suaidy et al (56) found that high moisture of 45 percent and freezing improved the milling performance of grain and resulted in flour with a higher extraction rate and a lower ash content than flour milled by customary procedures.

**PREPARATION AND PROCESSING OF GRAIN PRODUCTS:** The factors that influence nutrients in baked or processed products are type and amount of added ingredients, fat absorption, water absorption, temperature of baking or processing, and special treatments. Products made from wheat flour usually gain in nutrient content because of the contribution of nutrients from the added ingredients. In some instances, the processed products increase in a nutrient due to contact with metal. Zook et al (54) found the tin content of bread to be 6.2 and 6.8 ppm, respectively, for conventional and continuous-mix types of bread in comparison to the flour value of 3.5 ppm. Amounts of calcium and phosphorus in these two types of bread were greater than in the flour used in their preparation (Table 3). These higher amounts may be attributed to the contribution of milk and the calcium salts used as dough improvers in the formulation (12).

Higher pentosan content was found in continuous-mix breads than in conventional-type breads (57). Breads made by the conventional-mix process were slightly higher in vitamin B<sub>6</sub> content than those made by the continuous-mix process; this was probably due to the variation in formulation (58). Higher potassium values for conventional-type breads can be attributed to the level of potassium bromate used in the formulation. Fat and fatty acid contents of breads depend on the amount and kind of fat used in the formulation (59).

The calcium and phosphorus contents of cake were much higher than those of the flour (53); this can be attributed largely to the phosphates from the leavening (Table 3). Cakes pick up the trace element nickel quite readily from the shortening ingredient as shown by reported values of 0.2 ppm for cake flour and 0.6 ppm for the baked cake (60). There was six times as much copper in the cake as in the flour (54).

Semolina and macaroni are very similar in their nutrient contents (Table 3) because only water is added in the preparation of the finished product. Limited studies have been reported on nutrient composition of pasta products. Many types of pasta on the market today are made with all semolina but others have the semolina diluted with farina or flour. These changes in formulation certainly affect the nutritive values of the raw product, change the water absorption level with cooking, and thus have a marked influence on nutritive values of the cooked product. The type and amount of egg solids used in noodles have a significant influence on lipid, fatty acid, and cholesterol content of the food. That is, whether whole eggs, egg yolks, or a blend of whole eggs and egg yolks are used in noodle formulations and the variations in the amounts used can cause marked influences on lipid, fatty

acid, and cholesterol content of the food. That is, whole eggs, egg yolks, or a blend of whole eggs and egg yolks are used in noodle formulations, and the variations in the amounts used can cause marked influences on lipid, fatty acid, and cholesterol, as well as on other nutrients.

What apparently seems to be only a slight variation in processing or preparation procedures of wheat products can cause marked changes in nutrients of the finished product. Cake doughnuts, for example, absorb more fat during frying than do raised doughnuts. This difference changes proximate composition, fatty acid content, total lipids, and other nutrients in the product. Low relative humidity and high temperatures in ovens during baking, as well as high pH (hydrogen-ion concentration) of the dough, can cause destruction of significant amounts of thiamin. The alkaline reaction needed for an appealing color in devil's food cake, for the optimum texture of saltines, or for the glaze on pretzels can result in extremely low values for thiamin in these finished products. The additional lye bath used in pretzel manufacturing may cause almost total destruction of the thiamin that was once present in the flour.

Retention of thiamin with baking of bread has been reported by Bottomley and Nobile (61) to be about 70 percent. Others have found an 80 percent retention of thiamin in bread baked at 246°C (475°F) for 30 min (62). Lower values for thiamin in breads have been reported for the crust than for the crumb (63). Auerman et al (64), in a comparison of riboflavin values, reported that breads made from flour of 72 to 85 percent extraction retained much less of their original riboflavin than did whole wheat breads during breadmaking. Little or no loss of niacin has been reported to be due to the baking process (65, 66). About 70 percent of the folacin is retained in bread with baking (52) and about 81 percent of the vitamin B<sub>6</sub> (41).

#### PROBLEMS TO BE SOLVED

Problems of several types are encountered in assembling data from published and unpublished sources for the Nutrient Data Bank (NDB) and for developing values for revision of Agriculture Handbook 8, which contains tables of food composition.

These problems include description of samples, terminology, methodology, and reporting of the results.

**DESCRIPTION OF SAMPLES:** Nutrient data of foods are often reported for samples that are inadequately described. A full description of the food is essential for adequate evaluation of the data. Details of treatment, production, processing, cooking methods, physical state, portion form in which data are reported (i.e., dry basis, fresh basis, percent of ash, etc.), and other factors influencing nutrient composition would be helpful.

**TERMINOLOGY:** In the cereal milling and processing industries, the terminology used to describe the products should have uniform meaning. Uniform or standard terminology is of special importance to those responsible for developing data on the composition of cereal products and for coding and recording accurately for the Nutrient Data Bank. Close cooperation with all

segments of the wheat industry is essential and will help to avoid errors in product classification in the future. Assistance of wheat millers and members of the baking industry is needed to continue to develop standard terminology and expand the code.

METHODOLOGY: The need for reliable standardized methods of analysis is acute for many foods and nutrients. Standard methods for quantitative analysis of many nutrients were developed for clinical materials such as blood, urine, tissues, pharmaceuticals, or standard solutions. These methods frequently need to be modified for successful adaptation to food analysis. Methods that work well for one food may need major adjustment for another food or food mixture. Evaluation of the method is particularly important if the food contains nutrients at levels near the limit of detectability. Standard methods adopted by the AACC, AOAC, and AOCS often need to be adapted for certain foods.

Of particular importance in methodology is preparation of the samples. Establishing appropriate procedures for hydrolysis of samples is probably the most important research problem yet to be solved in determining amino acid content of food. Vitamin analysis by microbiological and spectrofluorometric methods is also a problem as well as the development of proper extraction methods for grain lipids.

Methodology is of concern also in mineral element determinations. This is illustrated by data for magnesium on the same samples of wheat and flour as analyzed by different methods (Table 4). The values determined by atomic absorption spectroscopy (54) were consistently higher than those determined by a chemical method (12). The latter method precipitates magnesium as magnesium ammonium phosphate from the filtrate after calcium is precipitated as the oxalate. If any magnesium was carried down with the calcium oxalate, the subsequent determination of magnesium would give low values, and calcium values would be high. There may be another explanation, but such differences in results from analyses of the same samples by different methods need to be resolved.

Data from published and unpublished sources will be entered in the NDB, and all factors such as type of wheat, cultivar, location of growth, etc., will be recorded whenever they are available. Data can be retrieved according to these factors. Some measure of variance in nutritive values will be available from the NDB. The revised Handbook 8 will present values for nutrients according to mean, standard error, and number of samples.

REPORTING RESULTS: One of the problems in interpreting data for cereal products concerns the procedure for arriving at the figure for protein content. In converting Kjeldahl nitrogen to protein content, the common practice has been to use 6.25 as the factor for all cereals but wheat, and this practice contains avoidable error. The factors developed by Jones in 1931 (67) are in good accord with earlier work by Osborne (68) and with more recent work by Tkachuk (69, 70). These factors for calculating protein from content of nitrogen take into account the nitrogen content of the principal protein or proteins present in the particular kind of food. According to Jones, the appropriate factors for wheat products are: Wheat grain, 5.83;

wheat bran, 6.31; wheat embryo, 5.80; and wheat endosperm, 5.70. The appropriate conversion factors should be used, and the factors used should always be reported in tables of data for protein content of foods.

Another problem in reporting results of nutrient analyses involves the carbohydrate content of foods. Values in the tables of food composition for total carbohydrate are presently determined by calculating the difference between the sum of the protein, fat, ash, and moisture content and 100 percent. When data for total carbohydrate are reported, they should include fiber. Often, however, fiber has been deducted from the carbohydrate value reported. For precise determinations of carbohydrate, the food should be subjected to direct analyses for starch, sugars, fiber, and other carbohydrate fractions. Future research could well be directed toward developing accurate and reproducible methods for individual carbohydrates and carbohydrate fractions. The laymen's and the medical profession's increased interest in fiber in diets for possible prevention of diseases of the colon and in the use of increased starch for diabetic diets makes these analyses for carbohydrate fractions extremely important.

#### COOPERATION NEEDED

At present, we anticipate further cooperation from industry in providing data for the NDB and for the revision of Agriculture Handbook 8. So far, data have been provided for flours, breads, cookies, crackers, toaster pastries, cakes, cake mixes, frozen waffles, muffin mixes, pizza, and a variety of pasta products including egg noodles, macaroni, and spaghetti. Many more values are needed for those and a variety of other foods so that those published will reflect normal variation in nutrient content. We urge your participation and cooperation now and in the future so that the most meaningful nutritive values can be reported, giving due weight to variation caused by customary growing, milling, baking, and processing practices.

#### ACKNOWLEDGMENT

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### Literature Cited

1. Watt, B. K. and Merrill, A. Composition of Foods--raw, processed, prepared. U.S. Dept. Agr. Handb. No. 8, 190 pp. (1963).
2. Schrenk, W. G. Minerals in wheat grain. Kans. Agr. Expt. Sta. Tech. Bul. 136, 23 pp. (1964).
3. Whitney, D. E., Herren, H., and Westerman, B. D. The thiamin and riboflavin content of the grain and flour of certain varieties of Kansas-grown wheat. Cereal Chem. 22: 90 (1945).
4. Berry, C. P., D'Appolonia, B. L., and Gilles, K. A. The characterization of triticale starch and its comparison with starches of rye, durum, and HRS wheat. Cereal Chem. 48: 415 (1971).
5. Mattern, P. J., Salem, A., Johnson, V. A., and Schmidt, J. W. Amino acid composition of selected high-protein wheats. Cereal Chem. 45: 437 (1968).
6. Middleton, G. K., Bode, C. E., and Bayles, B. B. A comparison of the quantity and quality of protein in certain varieties of soft wheat. Agron. Jour. 46: 500 (1954).
7. Tkachuk, R. and Irvine, G. N. Amino acid compositions of cereals and oilseed meals. Cereal Chem. 46: 206 (1969).
8. Eheart, J. F. and Mason, B. S. Nutrient composition of selected wheats and wheat products. V. Carbohydrate. Cereal Chem. 47: 715 (1970).
9. Weihrauch, J. L., Kinsella, J. E., and Watt, B. K. Comprehensive evaluation of fatty acids in foods. V. Cereal products. (In press).
10. Slover, H. T., Lehmann, J., and Valis, R. J. Nutrient composition of selected wheats and wheat products. III. Tocopherols. Cereal Chem. 46: 635 (1969).
11. Hepburn, F. N. Nutrient composition of selected wheats and wheat products. VII. Total and free niacin. Cereal Chem. 48: 369 (1971).
12. Hepburn, F. N. and Tulloss, J. H. Nutrient composition of selected wheats and wheat products. IX. Contents of ash, iron, phosphorus, calcium, magnesium, potassium, and sodium. (In press).
13. O'Donnell, W. W. and Bayfield, E. G. Effect of weather, variety, and location upon thiamin content of some Kansas-grown wheats. Food Res. 12: 212 (1947).

14. Basargin, N. N. and Peregudova, L. A. Zinc and molybdenum content of different varieties of spring and winter wheat. *Voprosy Pitaniya* 28: 65 (1969).
15. Basargin, N. N. and Peregudova, L. A. Copper and manganese content in different varieties of spring and winter wheat. *Voprosy Pitaniya* 27: 78 (1968).
16. Deosthale, Y. G.; Suryanarayana Rao, K., and Mohan, V. S. Nutritive value of some varieties of wheat. *Indian Jour. Nutr. and Dietet.* 6: 182 (1969).
17. Shoup, F. K., Pomeranz, Y., and Deyoe, C. W. Amino acid composition of wheat varieties and flours varying widely in bread-making potentialities. *Jour. Food Sci.* 31: 94 (1966).
18. Shuey, W. C. and Gilles, K. A. Evaluation of durum wheat and durum products. I. Studies in semolina and macaroni with the amylograph. *Cereal Chem.* 41: 32 (1964).
19. Miller, B. S., Seiffe, J. Y., Shellenberger, J. A., and Miller, G. D. Amino acid content of various wheat varieties. I. Cystine, lysine, methionine, and glutamic acid. *Cereal Chem.* 27: 96 (1950).
20. Schrenk, W. G. and King, H. H. Composition of three varieties of Kansas-grown wheat. Mineral analysis of wheat and soil. *Cereal Chem.* 25: 61 (1948).
21. King, H. H. and Perkins, A. T. Plant nutrition investigation. Kans. Agr. Expt. Sta. Rpt. 1932-34, p. 28 (1934).
22. Kuhn, H. and Schaumloffel, E. Effect of high copper rates in cereal growth. *Landwirtsch. Forsch.* 14: 82 (1961).
23. Shellenberger, J. A. and Schrenk, W. G. Mineral analysis of Peruvian wheat. *Cereal Chem.* 25: 407 (1948).
24. Skarsaune, S. K., Youngs, V. L., and Gilles, K. A. Changes in wheat lipids during seed maturation. I. Physical and chemical changes in the wheat kernel. *Cereal Chem.* 47: 522 (1970).
25. Skarsaune, S. K., Youngs, V. L., and Gilles, K. A. Changes in wheat lipids during seed maturation. II. Changes in lipid composition. *Cereal Chem.* 47: 533 (1970).
26. Abernethy, R. H., Paulsen, G. M., and Ellis, R., Jr. Relationship among phytic acid, phosphorus, and zinc during maturation of winter wheat. *Jour. Agr. and Food Chem.* 21: 282 (1973).

27. Abou-Guendia, M. and D'Appolonia, B. L. Changes in carbohydrate components during wheat maturation. II. Changes in sugars, pentosans, and starch. *Cereal Chem.* 50: 723 (1973).
28. Cerning, J. and Guilbot, A. Changes in the carbohydrate composition during development and maturation of the wheat and barley kernel. *Cereal Chem.* 50: 220 (1973).
29. Dubetz, S. Effects of nitrogen on yield and protein content of Manitou and Pitic wheats grown under irrigation. *Canadian Jour. Plant Sci.* 52: 887 (1972).
30. Singh, D. P. and Sharma, H. C. The effect of different doses of nitrogen, phosphorus, and potash on the growth, yield, and quality of wheat. *Beitrage zur Tropischen und Subtropischen Landwirtschaft und Tropenveterinarmedizin* 10: 315 (1972).
31. Johnson, V. A., Dreier, A. F., and Grabouski, P. H. Yield and protein responses to nitrogen fertilizer of two winter wheat varieties differing in inherent protein content of their grain. *Agron. Jour.* 65: 259 (1973).
32. Ghanbari, H. A. and Mameesh, M. S. Iron, zinc, manganese, and copper content of semidwarf wheat varieties grown under different agronomic conditions. *Cereal Chem.* 48: 411 (1971).
33. Fine, L. O. Mineral content of South Dakota bread wheats: extent and nature. *Agron. Jour.* 64: 769 (1972).
34. Hunter, A. S. and Stanford, G. Protein content of winter wheat in relation to rate and time of nitrogen fertilization application. *Agron. Jour.* 65: 772 (1973).
35. Abrol, Y. P., Uprety, D. C., Ahuja, V. P., and Naik, M. S. Soil fertilizer levels and protein quality of wheat grains. *Australian Jour. Agr. Res.* 22: 195 (1971).
36. Hojjati, S. M. and Maleki, M. Effect of potassium and nitrogen fertilization on lysine, methionine, and total protein contents of wheat grain, *triticum aestivum* L. em. Thell. *Agron. Jour.* 64: 46 (1972).
37. Gately, T. F. Effect of N fertilizer on the yield and nitrogen content of spring wheat (cultivar Quern) in 1967 and in 1969. *Irish Jour. Res. Agr. Res.* 10:323 (1971).
38. Greaves, J. E. and Heist, C. T. The mineral content of grain. *Jour. Nutr.* 1: 293 (1929).

39. Greaves, J. E. and Nelson, D. H. The iron, chlorine, and sulfur contents of grains and the influence of irrigation water on them. *Soil Sci.* 19: 325 (1925).
40. Greaves, J. E. and Heist, C. T. The phosphorus of grains. *Cereal Chem.* 6: 115 (1929).
41. Polansky, M. M. and Toepfer, E. W. Effect of fumigation on wheat in storage. III. Vitamin B<sub>6</sub> components of wheat and wheat products. *Cereal Chem.* 48: 392 (1971).
42. Shuey, W. C. and Gilles, K. A. Milling evaluation of hard red spring wheats. V. Relation of wheat proteins, wheat ash, bran pentose, flour pentose, and starch on bran to milling results. *Cereal Chem.* 50: 37 (1973).
43. Tkachuk, R. Amino acid composition of wheat flours. *Cereal Chem.* 43: 207 (1966).
44. Hepburn, F. N., Calhoun, W. K., and Bradley, W. B. The distribution of the amino acids of wheat in commercial mill products. *Cereal Chem.* 39: 749 (1960).
45. Tara, K. A. and Bains, G. S. Effect of milling on the lysine content of flour of fortified wheat. *Indian Jour. Nutr. and Dietet.* 9: 206 (1972).
46. Waggle, D. H., Lambert, M. A., Miller, G. D., Farrell, G. P., and Deyoe, C. W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. *Cereal Chem.* 44: 48 (1967).
47. Czerniejewski, C. P., Shank, C. W., Bechtel, W. G., and Bradley, W. B. The minerals of wheat, flour, and bread. *Cereal Chem.* 41: 65 (1964).
48. Ferretti, R. J. and Levander, O. A. Effect of milling and processing on the selenium content of grains and cereal products. *Jour. Agr. and Food Chem.* 22: 1049 (1974).
49. Hepburn, F. N. Nutrient composition of selected wheat and wheat products. VIII. Contents of thiamin and riboflavin. *Cereal Chem.* (In press).
50. Pomeranz, Y., ed. *Wheat Chemistry and Technology*. 2nd ed. American Association of Cereal Chemists. St. Paul, MN. 821 pp. (1971).
51. Schultz, A. S., Atkin, L., and Frey, C. N. The vitamin B<sub>1</sub> content of wheat, flour, and bread. *Cereal Chem.* 16: 643 (1939).

52. Butterfield, S. and Calloway, D. H. Folacin in wheat and selected foods. Amer. Dietet. Assoc. Jour. 60: 310 (1972).
53. Toepfer, E. W., Polansky, M. M., Eheart, J. F., Slover, H. T., Morris, E. R., Hepburn, F. N., and Quackenbush, F. W. Nutrient composition of selected wheats and wheat products. XI. Summary. Cereal Chem. 49: 173 (1972).
54. Zook, E. G., Greene, F. E., and Morris, E. R. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, zinc, magnesium, lead, tin, cadmium, chromium, and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem. 47: 720 (1970).
55. Kulp, K. Pentosans of wheat endosperm. Cereal Sci. Today 13: 414 (1968).
56. Al-Suaidy, M. A., Johnson, J. A., and Ward, A. B. Effects of certain biochemical treatments on milling and baking properties of hard red winter wheat. Cereal Sci. Today 18: 174 (1973).
57. D'Appolonia, B. L. Comparison of pentosans extracted from conventional and continuous bread. Cereal Chem. 50: 27 (1973).
58. Polansky, M. M. and Toepfer, E. W. Nutrient composition of wheat and wheat products. IV. Vitamin B<sub>6</sub> components. Cereal Chem. 46: 664 (1969).
59. Fleishman, A. I., Eastwood, G., and Davis, M. Total lipids and fatty acids in bread. Amer. Dietet. Assoc. Jour. 43: 537 (1963).
60. Matthews, R. H., Weihrauch, J. L., and Watt, B. K. Nutrient content of wheat and rice. Cereal Foods World 20: 348 (1975).
61. Bottomley, R. A. and Nobile, S. The thiamine content of flour and white bread in Sydney, New South Wales. Jour. Sci. Food and Agr. 13: 550 (1962).
62. Coppock, J. B. M., Carpenter, B. R., and Knight, R. A. Thiamine losses in bread baking. Chem. Indus. [London] 23: 735 (1957).
63. Morgan, A. F. and Frederick, H. Vitamin B (B<sub>1</sub>) in bread as affected by baking. Cereal Chem. 12: 390 (1935).
64. Auerman, L. J., Bukin, V. N., Zajceva, Z. I., Kuceva, L. S., and Pasouskin, V. F. The preservation and content of vitamin B<sub>1</sub>, B<sub>2</sub>, and PP in bread from different sorts of flour. Biochem. Lerna 2: 193 (1954).

65. Sherwood, R. C. Accomplishment in cereal fortification. Amer. Jour. Public Health 33: 526 (1943).
66. Menden, E. and Cremer, H. D. The problem of improving nutritive value with special reference to enrichment of food. Food Manuf. 34: 65 (1959).
67. Jones, D. B. Factors for converting percentage of nitrogen in foods and feeds into percentage of protein. U.S. Dept. Agr. Cir. 183, 22 pp. Sl. rev. 1941, 22 pp. (1931).
68. Osborne, T. B. The proteins of the wheat kernel. Carnegie Inst. Pub. No. 84 (1907).
69. Tkachuk, R. Note on the nitrogen-to-protein conversion factors for wheat flour. Cereal Chem. 43: 223 (1966).
70. Tkachuk, R. Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem. 46: 419 (1969).

### TOTAL LIPIDS in WHEAT

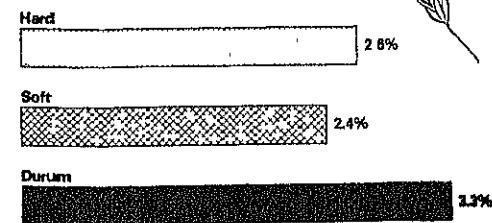


Figure 1. Total lipid content of three types of wheat.

### ALPHA TOCOPHEROL in WHEAT

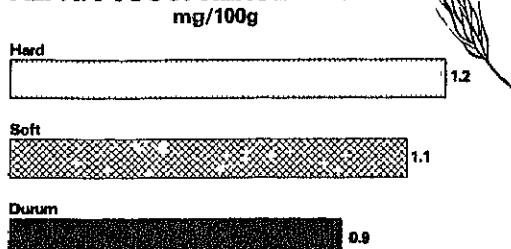


Figure 2. Alpha tocopherol content of three types of wheat.

### PHOSPHORUS in WHEAT

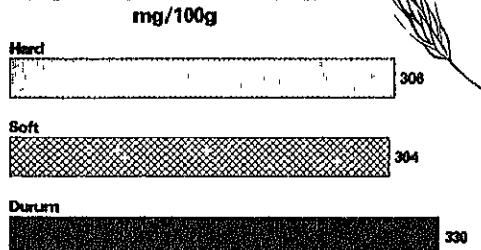


Figure 3. Phosphorus content of three types of wheat.

### MAGNESIUM in WHEAT

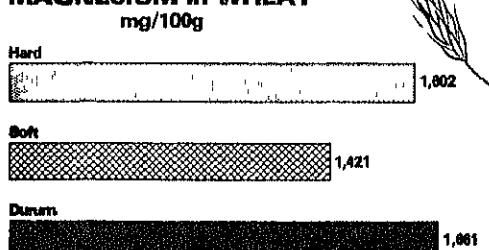


Figure 4. Magnesium content of three types of wheat.

### IRON, CALCIUM, PHOSPHORUS, POTASSIUM in Wheat Grain, Flour, and Semolina

	IRON	CALCIUM	PHOSPHORUS	POTASSIUM
	mg/100g	mg/100g	mg/100g	mg/100g
Hard wheats	4.0	33	306	388
Flour	.7	17	90	99
Soft wheats	3.3	36	304	442
Flour	.7	17	74	106
Durum wheats	3.6	30	330	441
Semolina	1.2	16	157	171

Table 1. Iron, calcium, phosphorus, and potassium content of wheat grain, flour, and semolina.

### ZINC, MAGNESIUM, NICKEL in Wheat Grain, Flour, and Semolina

	ZINC	MAGNESIUM	NICKEL
	ppm	ppm	ppm
Hard wheats	21.4	1,802	0.42
Flour	5.4	292	.13
Soft wheats	19.3	1,421	.28
Flour	3.4	194	.16
Durum wheats	27.0	1,861	.26
Semolina	8.2	589	.15

Table 2. Zinc, magnesium, and nickel content of wheat grain, flour, and semolina.

### CALCIUM, PHOSPHORUS, POTASSIUM in Flours, Semolina and Their Products

	CALCIUM	PHOSPHORUS	POTASSIUM
	mg/100g	mg/100g	mg/100g
Flour (bread)	17	80	89
Bread-conventional	96	95	127
Bread-continuous-mix	102	113	87
Flour	17	74	105
Cake	63	235	110
Semolina	18	157	171
Macaroni	18	166	188

Table 3. Calcium, phosphorus, and potassium content of flours, semolina, and their baked or processed products.

### MAGNESIUM In Wheat Grain and Flour

METHOD	WHEAT GRAIN	PATENT FLOUR
	mg.	mg.
Atomic absorption *	160	29
Chemical *	139	22

Customary moisture content: \* Zook et al; \* Hepburn

Table 4. Magnesium content of wheat grain and flour determined by two methods.

## WHEAT FIBER IN THE HUMAN DIET<sup>1</sup>

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The question might be raised as to why there has developed within the past few years such a concern about incorporating fiber into the human diet. This should be of particular interest to everyone who is involved with wheat or wheat products. Practically all references to foods that are rich in fiber specifically mention wheat bran. That product, according to tables of food composition, contains from 9 to 12 percent fiber (1, 2). As such, it is one of the major sources of fiber that can be included in the human diet. This information is not new; it has been recognized for over a hundred years and so does not explain the great interest that currently is developing in this area of medicine and nutrition.

It is possible that the early espousal of the role of fiber in the diet by the so-called food faddists led the more conservative investigators to turn away from that topic. Furthermore, since fiber in the diet could not be utilized even as a source of energy (the lowest dietary contribution in the minds of many nutrition scientists) and the amount in most foods was relatively small, there appeared to be little reason for spending any research time or effort on that topic. According to Southgate (3), the fiber in the diet of American children might contribute 1 percent to their energy intake if it were available. For adults in Scotland, the maximum caloric contribution from fiber, again if it were available, would be 4 percent.

Early in the development of one segment of the American food industry, bran became the popular means of maintaining regularity. As such, it became an important component of the dietary programs advocated by such health proponents as Kellogg. In his writings, Kellogg (4) claimed that constipation resulted in "auto-intoxication or intestinal toxemia." This, in turn, had an adverse effect on the lower intestinal tract. Kellogg went on to state that "It is quite possible that more human suffering, physical and mental, has resulted from...constipation and its sequelae." Is it possible that Kellogg was prescient enough to realize what would appear in the medical literature only 20 years after his death? Reviewing the vast number of papers that have appeared in the past decade, specifically mentioning bran, makes it look as though many of the diseases to which we Americans are prone, in some way, may be related to the reduction in the fiber content of our diet. This situation has moved so rapidly that one of the activist groups has petitioned, by means of a "white paper," a number of federal agencies to encourage the consumption of those foods known to be rich in fiber such as "whole grain foods" (5).

One of the first problems confronting us today is the nature of the compounds included in the general term dietary fiber. By definition and the precedence established by the A.O.A.C. (6), this group of substances is considered to be resistant to the hydrolytic processes occurring in the human gastrointestinal tract. For that reason, one of the oldest methods of their estimation involved the hydrolysis of a food with dilute acid, followed by the same treatment with dilute alkali. The dried residue was corrected for any inorganic material by ashing it in a furnace. This procedure is essentially the same as

that proposed by Heinrich Einhof, a German chemist, who, about 1806, used the method for estimating the nutritive value of various plant products (7).

The residue so measured is a mixture of different carbohydrate-like substances and some that are not so related. Besides cellulose, hemicellulose (complex polymers of xylose, arabinose, glucuronic acid, galactose, mannose and occasionally other sugars, (7), pectin (composed primarily of galacturonic acid, arabinose and galactose, (7), gums and mucilages, this group also includes the lignins which are polymers of phenylpropane. To further complicate the picture, none of these names refers to single, discrete compounds; each is really a group name encompassing a fairly large series of compounds which differ only slightly from each other. For these and other reasons, a great deal of work remains to be done, especially in the development of analytical methods, before it will be possible to separate facts from the theories that have become associated with this segment of nutrition.

The fibrous material in the human diet presumably represents the non-digestible organic material. Unlike ruminants, man is characterized by not having those microorganisms in his intestinal tract which can degrade cellulose and related substances to a form which can be absorbed. Whether this is an absolute deficiency has not been resolved at this time. The ultimate test may require a comparison of the same fibrous foods, since *in vitro* studies suggest that even in ruminants, the digestibility of the fibrous material of different plants varies. According to Van Soest and McQueen (7), *in vitro* studies indicate that the fibrous material in cauliflower, rutabagas, potatoes, carrots and apples is digested to the extent of 80 to 93 percent; whereas only 33 percent of that in wheat bran is digested.

Although no one individual is responsible for refocusing attention on the fiber in the diet, considerable credit goes to Dr. Denis Burkitt. When Dr. Burkitt began to champion the cause of dietary fiber, he had achieved an outstanding place in medical research as a result of his work on the transmission of the lymphoma which bears his name. In a way, it was fortunate that he did so much of the spade work, for if a nutrition scientist had made the same suggestions, it is likely that his observations would have been ignored by most of the scientific world.

Burkitt's report attracted considerable attention because it suggested that the increasing incidence of colonic cancer in western countries was associated with a decreasing intake of dietary fiber (8). Deaths from colonic cancer in this country, for both males and females, is the second most frequent cause of cancer mortality. It, like lung cancer, appears to have increased markedly over the past 50 years in the developed countries, while among the rural natives in most of the underdeveloped regions, it is practically unknown. When inhabitants from areas with a low incidence of colonic cancer migrate to metropolitan areas or to western countries, mortality from this neoplasm increases (9). In all of these situations, the epidemiological evidence suggests an inverse relation between the mortality rate and the intake of dietary fiber.

The epidemiological data for large numbers of people when broken down into smaller segments suggest some peculiarities which were not apparent in earlier reports. This is true for the incidence (presumably morbidity) of colonic cancer. According to Macdonald (10), the age adjusted incidence rate among

caucasian males ranged from 14.6 per 100,000 in Nevada and El Paso, Texas to 26.7 in Connecticut and Houston, Texas. A similarly wide deviation exists in Canada where the rate is 14.0 in Alberta, while in neighboring Saskatchewan, the rate is 27.6. Unfortunately, in these regional compilations little, if any, attention has been paid to the diets followed by the people. However, Macdonald (10) does refer to a study she performed in Texas. There, the less affluent Latin Americans used corn as their dietary staple; "...their rates for all cancer..." (except of the cervix and lung among females) "...are lower than the rates among Negroes and non-Latin Caucasians in the same community."

One of the major factors associated with the decreased incidence of colonic cancer among individuals who routinely consume a diet containing large amounts of fiber is the rapid transit of food through the gastrointestinal tract. When Burkitt, Walker and Painter (11) graphed the transit time against the weight of the stools, they found that for some individuals it required as long as 140 hours for the food to traverse the entire gastrointestinal tract. For those individuals, the weight of the stools was about 100 g per day. Other individuals with stool weights around 400 to 550 g per day, had transit times of from 20 to 40 hours. There was considerable spread especially among the intermediate values. The largest stools and most rapid transit times were observed among the inhabitants of those regions where diseases of the lower part of the intestinal tract were almost unknown. Confirmation for this association has come from clinical studies of individuals whose diets initially contained only minimal amounts of fiber. These subjects had prolonged transit times. By adding "two heaped dessertspoonfuls (approximately 10 g) of unprocessed millers' wheat bran" to their daily diet, Payler and Coworkers (12) were able to reduce the time required for the ingested radiopaque pellets to appear in the feces.

The decreased transit time associated with the ingestion of diets containing large amounts of fiber may influence the interaction of the fiber and bile acids. One of these interactions involves the physical changes associated with the presence of fiber in the colonic contents. In vitro studies by Kritchevsky and Story (13) indicate that the fiber in wheat straw, bran, alfalfa and similar substances binds bile salts and presumably makes them unavailable for absorption. It is possible that this binding of bile acids may also remove them from the sphere of bacterial action. It has been postulated (14) that the numbers and kinds of bacteria populating the colon play a decisive role in the formation of carcinogens and/or co-carcinogens. These compounds may be formed from either dietary components or from intestinal secretions. The latter are presumably bile acids. The secretion of bile acids is increased in proportion to the level of dietary fat. Diets that contain large amounts of fiber are usually low in fat; for that reason, the amount of bile acids secreted into the intestine is correspondingly reduced. The high fiber diet favors the development of a colonic microflora that is less likely to act on bile acids and other precarcinogenic compounds than is the case where the diet contains little fiber. This was confirmed by studies of the fecal material from subjects in India, Uganda and Japan. There, where colonic cancer is rare, the amount of fecal bile acids and their degradation products was low. Drasar and Hill (14) also pointed out that the change in the microfloral situation in the colon might reduce the formation of co-carcinogens from amino acids such as tyrosine and tryptophan.

Additional evidence that the presence of bran in the diet alters the activity of the microflora in the lower part of the intestinal tract comes from the report of Pomare and Heaton (15). They found that supplementing the diets of normal women with 33 g of unprocessed bran per day produced a reduction in their bile of those derivatives which have been associated with the development of colonic cancer. Their paper points out that the compounds which were present in low concentration during bran supplementation, when injected into rats produced colonic carcinomas. The beneficial effect of the bran on the presence of potentially carcinogenic compounds was ascribed to the possibility that the increased mass of material in the colon may have prevented "physical contact between bile salts and colonic bacteria." Pomare and Heaton (15) stated that the changes they observed in the composition of bile from their subjects while consuming bran are similar to those present in individuals from those areas of the world where colonic cancer is rare.

Another disease for which wheat bran may become a regular part of the treatment is diverticulosis of the colon. This condition, which has increased in both incidence and severity of symptoms over the past century was formerly treated with a bland, soft diet. According to Painter and Coworkers (16), this treatment has been followed for 50 years "despite the lack of any convincing evidence of its beneficial results." Since Painter and Burkitt (17) suggested that diverticulosis was associated with a reduction in dietary fiber, it became imperative to test the therapeutic effect of a high fiber diet for patients with this disturbance. Seventy patients with diverticular disease were advised to eat a diet containing whole meal bread, fruit and vegetables supplemented with two teaspoons of unprocessed bran three times a day; after two weeks, the bran intake was increased if necessary to produce daily bowel movements which required no "straining" (16). After 22 months, 62 patients had marked improvement in their symptoms. Eight did not tolerate the bran, but three of these were so improved by the basal, high fiber diet, that they continued the regimen without the bran.

Of perhaps greater interest, are the reported effects of bran on cardiovascular disease. Although there is no absolute relation between serum cholesterol levels and incidence of certain cardiovascular disturbances, most investigations suggest an increased risk of attack whenever the serum cholesterol level is elevated. It has been pointed out by Hellendoorn (18) that the diets of people who seldom develop ischemic heart disease are not only low in fat but also high in "residue." He refers to the work of Antonis and Bersohn (19) who varied the amount of fiber in the diets of South African white and Bantu prisoners. Although Hellendoorn (18) claimed that decreasing the fiber content of these diets increased the serum cholesterol levels, the original paper (19) in summarizing the results stated that "Serum lipid concentrations do not appear to be affected by the quantity of fiber in the diet."

Differences in serum cholesterol levels were observed among vegetarians and non-vegetarians (20). These differences were also related to the fiber content of their diets. On the basis of calculations using tables of food composition, the daily fiber intake of the strict vegetarian adults was estimated to be 20 to 24 g; 13 to 16 g for the lacto-ovo-vegetarians and 8 to 11 g for the non-vegetarians (21). The serum cholesterol levels of these adults were 206, 256 and 291 mg per 100 ml for the vegetarians, the lacto-ovo-vegetarians and the non-vegetarians respectively.

Although there are additional reports suggesting a beneficial effect of high fiber diets as protective regimens against cardiovascular disease, there is not complete agreement in this area. A number of investigators have confirmed the report of Antonis and Bersohn (19) which indicated that dietary fiber has no effect on serum cholesterol levels. One of these is Eastwood (22), who claimed that "...increasing the cereal-fibre content of the diet itself does not reduce the serum-lipid concentration..." That conclusion was based on a study of a group of lacto-ovo-vegetarian monks. The diet for half of the monks was altered by substituting "white, high extraction bread" for their regular whole meal bread. This reduced their intake of fiber from an average of about 23 g down to 18. For the other group of monks, the fiber intake was increased to 37 g per day by means of biscuits to which fiber had been added. The serum cholesterol level decreased among the monks who were under 40 years of age and for whom the dietary fiber level was reduced. The reduction was from 250 mg per 100 ml to 186 at the eighth week. In the next four weeks, the concentration of serum cholesterol increased to 227 mg despite no change in the diet.

The effect of dietary fiber on serum cholesterol levels may continue to be a controversial matter. A solution to the question may not be forthcoming since so many factors appear to influence the level of this serum constituent. The resolution of this enigma may be of more academic than practical importance since the people in those regions of the world where ischemic heart disease is relatively rare consume diets that are high in crude fiber and low in fat. This relationship between these two dietary components exists wherever relatively unprocessed foods are the primary components of the diet.

The overwhelming attention recently focused on the role of dietary fiber has overshadowed the considerations that should be given to other wheat components. The starch in wheat is a good example since it may function in somewhat the same way as fiber, at least in certain physiological reactions. This was evident in our study of normal young men fed a diet which provided them with 90 to 95 percent of their protein from wheat. To do that, these subjects consumed 24 slices of white bread per day. The latter was associated with a marked increase in daily stool weights. While the subjects consumed a typical American diet restricted to 70 g protein per day, their stool weights averaged 80 g. As soon as the "bread" diet began, stool weights rapidly increased to 160 g by the fourth week. Thereafter, the weight of the stools decreased, but even after 50 days on the bread diet, their weights were significantly above those of the pre-bread control period (23).

It is doubtful that the observed change in stool weights among our subjects can be explained on the basis of dietary fiber. Calculations of the fiber content of the two diets showed only a minor increase in fiber content of the "bread" diet. This insignificant difference arises from the fact that the 70 percent extraction flour used in making the bread contains a very small amount of fiber. On that basis, we think that components in wheat, other than dietary fiber, may be important in determining the nature of the food residue in the lower part of the intestine. One might propose that since there is a correlation between the bulkiness of the stool and transit time, the larger stools during the bread period might have been associated with a more rapid movement of the food residue through the intestinal tract. On that basis, we might postulate that the much maligned white bread may have some of the beneficial health effects so frequently attributed solely to whole wheat breads presumably used in large amounts during the "good old days" when hard physical labor was

the fate of most men and women, and for such caloric expenditure they received only enough money to purchase little besides bread.

The other area where starch may play an important health role is in regulating the level of cholesterol in the blood. Only minor attention has been paid to this area since so many investigators assume that starch is starch. The work of Groen and collaborators (24) indicates that the inclusion of large amounts of bread (425 g/day) in the diets of 17 healthy Israeli subjects was associated with a reduction of serum cholesterol from 225 mg per 100 ml to 170 mg.

Another paper by de Groot and colleagues (25) suggests hypocholesterolemic effects of starches from different cereals. They reported that oat starch was slightly more effective in this respect than wheat when incorporated into bread fed 21 men. The greater reduction in serum cholesterol levels during the oat bread period occurred despite a lower fiber content of the diet than when whole wheat bread was fed.

In another publication (26), we have discussed the ability of large amounts of bread to aid overweight individuals in their "battle of the bulge" without any of the unpleasant sequelae so frequently experienced by many who follow the "7 day wonder diets." The wonder is how anyone could follow these diets for more than 7 days! Large amounts of bread in reducing diets obviates the development of ketosis with all its unpleasant effects; bread produces a comfortable feeling of satiety; it has a beneficial effect on serum lipids; and bread can be maintained in the diet after the desired body weight has been attained.

These are some of the reasons why the future of bread in both the gastronomic as well as health areas should make everyone affiliated with the wheat industry proud that their products may be the keystone to a better and more healthful life.

#### Literature Cited

1. Watt, B. K., and Merrill, A. L. Composition of Foods. Handbook No. 8, ARS, U. S. Department of Agriculture, Washington, D. C. (1963).
2. Church, C. F., and Church, H. N. Food Values of Portions Commonly Used. 11th ed. J. B. Lippincott Co., Philadelphia (1969).
3. Southgate, D. A. T., and Durnin, J. V. G. A. Calorie conversion factors. Br. J. Nutr. 24: 517-535 (1970).
4. Kellogg, J. H. The Itinerary of a Breakfast. Funk & Wagnalls Co. p. 65. New York (1920).
5. Community Nutrition Institute. Scientists urge more dietary fiber. CNI Weekly Report 4(31): 6 (Aug. 1, 1974).
6. A.O.A.C. Methods of Analysis. Crude Fiber. p. 129-131 (1970).
7. Van Soest, P. J., and McQueen, R. W. The chemistry and estimation of fibre. Proc. Nutr. Soc. 32: 123-130 (1973).

8. Burkitt, D. P. Epidemiology of cancer of the colon and rectum. *Cancer* 28: 3-13 (1971).
9. Stewart, H. L. Geographic pathology of cancer of the colon and rectum. *Cancer* 28: 25-28 (1971).
10. Macdonald, E. J. Epidemiology of colon-rectal cancer 1972. *Cancer Bulletin* March-April p. 33-41 (1973).
11. Burkitt, D. P., Walker, A. R. P., and Painter, N. S. Effect of dietary fibre on stools and transit-times, and its role in the causation of disease. *Lancet* 2: 1408-1412 (1972).
12. Payler, D. K., Pomare, E. W., Heaton, K. W., and Harvey, R. F. The effect of wheat bran on intestinal transit. *Gut* 16: 209-213 (1975).
13. Kritchevsky, D., and Story, J. A. Binding of bile salts in vitro by non-nutritive fiber. *J. Nutr.* 104: 458-462 (1974).
14. Drasar, B. S., and Hill, M. J. Intestinal bacteria and cancer. *Am. J. Clin. Nutr.* 25: 1399-1404 (1972).
15. Pomare, E. W., and Heaton, K. W. Alteration of bile salt metabolism by dietary fibre (bran). *Br. Med. J.* 4: 262-264 (1973).
16. Painter, N. S., Almeida, A. Z., and Colebourne, K. W. Unprocessed bran in treatment of diverticular disease of the colon. *Br. Med. J.* 2: 137-140 (1972).
17. Painter, N. S., and Burkitt, D. P. Diverticular disease of the colon: A deficiency disease of western civilization. *Br. Med. J.* 2: 450-454 (1971).
18. Hellendoorn, E. W. Physiological importance of indigestible carbohydrates in human nutrition. *Voeding* 34e: 618-635 (1973).
19. Antonis, A., and Bersohn, I. The influence of diet on serum lipids in South African white and Bantu prisoners. *Am. J. Clin. Nutr.* 10: 484-499 (1962).
20. Hardinge, M. G., and Stare, F. J. Nutritional studies of vegetarians. II. Dietary and serum levels of cholesterol. *J. Clin. Nutr.* 2: 83-88 (1954).
21. Hardinge, M. G., Chambers, A. C., Crooks, H., and Stare, F. J. Nutritional studies of vegetarians. III. Dietary levels of fiber. *Am. J. Clin. Nutr.* 6: 523-525 (1958).
22. Eastwood, M. Dietary fibre and serum-lipids. *Lancet* 2: 1222-1225 (1969).

23. Bolourchi, S., Friedemann, C. M., and Mickelsen, O. Wheat flour as a source of protein for adult human subjects. Am. J. Clin. Nutr. 21: 827-835 (1968).
24. Groen, J. J., Balogh, M., Yaron, E., and Freeman, J. Influence of the nature of the fat in diets high in carbohydrate (mainly derived from bread) on the serum cholesterol. Am. J. Clin. Nutr. 17: 296-304 (1965).
25. De Groot, A. P., Luyken, R., and Pikaar, N. A. Cholesterol-lowering effect of rolled oats. Lancet 2: 303-304 (1963).
26. Mickelsen, O. The nutritional value of bread. Cereal Foods World 20: 308-310 (1975).

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## RECOMMENDED FORTIFICATION POLICY FOR CEREAL-GRAIN PRODUCTS

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Two years ago at the Eighth National Conference on Wheat Utilization Research held in Denver I discussed the possible future look of cereal-grain enrichment programs. At that time the recommendations of the Panel of the Committee on Food Standards and Fortification Policy of the Food and Nutrition Board that was reviewing cereal-grain enrichment had not been published. The recommendations were published a little more than a year ago and have been under review since that time by several groups, including the technical groups of the major trade associations concerned with cereal-grain products.

For background information on how the Panel developed its recommendations, I recommend reading the publication of the National Academy of Sciences, entitled "Proposed Fortification Policy for Cereal-Grain Products", published in 1974, and also the presentation I made to this group two years ago, which was published in the Proceedings of that meeting.

By way of a brief review, the Panel recommended that cereal-grain products serve as an excellent carrier for nutrients to be supplied in fortification programs. This conclusion was based on the relative economy of cereal-grain products, their technical characteristics as a carrier for added nutrients, and the frequency and range of use in the diet. Cereal-grain products are consumed by approximately 98 percent of the U.S. population. These products contribute in excess of 25 percent of the average daily caloric intake. The cereal flour equivalent of cereal-grain products provides approximately 17 percent of the daily caloric intake in the United States. Therefore, most people do consume cereal-grain products of various kinds in significant amounts and essentially on a daily basis.

The recommendations of the Panel for the nutrients to be included in a cereal-grain fortification program and the level of nutrients to be provided in the cereal-grain flours are as listed below:

Nutrients Recommended for Fortification  
of Cereal-Grain Products

	<u>Mg/lb.</u>
Vitamin A	1.3
Thiamin	2.9
Riboflavin	1.8
Niacin	24.0
Vitamin B <sub>6</sub>	2.0
Folic Acid	0.3
Iron	40.
Calcium	900.
Magnesium	200.
Zinc	10.

I wish to emphasize that the levels of nutrients listed are those in the final cereal flour and not levels of nutrients recommended to be added to cereal flours. Thus these levels would be made up in the combination of nutrients native to the cereal flour and those nutrients added to achieve this recommended level.

The recommended level of vitamin A in this listing is at variance with the level originally published in the National Academy of Sciences report. The publication recommends 2.2 milligrams per pound of vitamin A based on retinol equivalents. Unfortunately, there was an error in the development of this level. This error resulted from the conversion of the 1968 RDA for vitamin A expressed in International Units to retinol equivalents, which is now the basis of recommending dietary allowances for vitamin A. The 1974 Food and Nutrition Board RDA's were not published at the time this report was issued. An unfortunate disparity in conversion from International Units to retinol equivalents occurred and resulted in the published level being higher than intended by the Panel. The 1.3 mg. per pound of retinol equivalents of vitamin A in cereal flour is consistent with the intent of the Panel, which felt that vitamin A should be supplied at a level of approximately twice the caloric contribution of the cereal flour. Since the cereal-grain products on a flour equivalent basis provided approximately 17 percent of the calories, the intent of the Panel was to provide approximately 35 percent of the RDA for vitamin A in its fortification recommendations. Products such as bread, which contain ingredients other than cereal flours, would provide nutrient levels proportional to the cereal content.

What proportion of the recommended dietary allowances for the ten nutrients recommended for inclusion in a cereal-grain fortification program would be provided if cereal-grain products were fortified as recommended? Based on cereal flours providing approximately 500 calories per day (17 percent of a daily caloric intake of 3,000 calories), the fortified cereal-grain products would contribute approximately the following levels of the RDA for the recommended nutrients:

**Contribution of Fortified Cereal-Grain Products  
to Recommended Dietary Allowances**

(1974 RDA for 19-11-year-old male)

Approximately 500 calories/day from Cereal-Grain Products  
(17% of caloric intake)

	% RDA
Vitamin A	35
Thiamin	50
Riboflavin	30
Niacin	30
Vitamin B6	35
Folic Acid	45
Iron	100
Calcium	30
Magnesium	15
Zinc	20

I am sure all of us can more readily understand the potential contribution of fortified cereal-grain products if these are translated in terms of specific foods. First let's look at the contribution that two slices of bread can make to the recommended dietary allowances of the 19-22-year-old male:

Nutrients Contributed by Common Cereal-Grain Products

<u>% of RDA</u>	<u>19-22-year-old male</u>
-----------------	----------------------------

1. 2 slices bread

	<u>Approx. % RDA</u>
Vitamin A	7.5
Thiamin	10.
Riboflavin	5.5
Niacin	6.5
Vitamin B <sub>6</sub>	5.5
Folic Acid	4.
Iron	22.
Calcium	6.
Magnesium	3.
Zinc	4.

Of course all population groups do not consume only wheat-base products as their primary cereal-grain source. Therefore I have also provided the contribution to the recommended dietary allowances of a serving of grits and a serving of rice:

2. Grits 1 oz. = 2/3 cup cooked

	<u>Approx. % RDA</u>
Vitamin A	8.0
Thiamin	12.
Riboflavin	7.
Niacin	7.
Vitamin B <sub>6</sub>	6.
Folic Acid	5.
Iron	25.
Calcium	7.
Magnesium	3.
Zinc	4.

3. Rice 1/4 cup = 1 cup cooked

	<u>Approx. % RDA</u>
Vitamin A	14.
Thiamin	20.
Riboflavin	11.
Niacin	13.
Vitamin B <sub>6</sub>	11.
Folic Acid	8.5
Iron	40.
Calcium	12.
Magnesium	6.
Zinc	7.

The Panel, as it considered this fortification policy, primarily looked at these recommendations on the basis of potential need for various population groups. It did not assess problems of technical feasibility. It is necessary to know, for example, the ability of the various cereal-grain products to carry the levels of nutrients recommended without significant separation in the manufacture and distribution process and without adverse impact on the flavor, appearance, baking or cooking, and keeping qualities of the products in which they are used. These are questions which must be answered by the industry working in conjunction with regulatory agencies and the nutrition community in general. As I indicated before, several of the technical groups of the cereal trade associations have been reviewing these recommendations for their impact on the above areas. I am sure that some problems are surfacing relative to the addition of some nutrients. This is the kind of input that will be ultimately required to arrive at a recommendation which will be compatible with the cereal-grain products and still provide a rational program for fortification of these products in relation to the needs of population groups.

The Panel also recommended that a common fortification program, where possible, be established for all cereal-grain products with enrichment standards. We felt this to be important, as people do consume (depending upon their individual preferences, ethnic origins, etc.) different cereal grains as the basic cereal in their diets. If all cereal flours are fortified in a similar manner, then the nutrient contribution from these cereals will be the same. It may not be possible, due to the nature of some cereal-grain products, to maintain this desired goal. Problems of color, of uniform distribution and impact on flavor or other product characteristics may result in reduced acceptance of the fortified product and thus negate the advantage of fortification.

The Panel recommended that the milling industry study the feasibility of retaining as much of the indigenous nutrient content as possible, consistent with producing a flour that would be suitable for making acceptable products from these cereal grains. We are still learning much about the contribution of some of the minor mineral elements to our physiological well being. We are also learning more about the value of some of the indigestible carbohydrate material in cereals and their value in our diets. Therefore, as a part of our research in evaluating fortification we should also look at the potential for modification of milling practices which may result in greater native nutrient retention.

The Panel was also concerned that, if and when these fortification recommendations were implemented, a procedure be established to assess the effectiveness of this program. We feel that this has been an important element which unfortunately has been missing from prior programs. In view of the cost of enrichment, and also in view of the purpose of enrichment being to improve the nutritional status of the American public, we certainly should have an assessment of its effectiveness. It is my personal hope that within the next year most of the technical evaluations concerning these recommendations can be carried out. It would then be possible to review the recommendations of

this Panel and determine what modifications, if any, were necessary to implement a fortification program for cereal-grain products based on an assessment of the need for nutrients in our diet, the feasibility of these nutrients to be provided through cereal-grain products, and the impact this program actually has on the nutritional well-being of the U.S. population.

## PHYTATE AS CARRIER FOR IRON - A BREAKTHROUGH IN IRON FORTIFICATION OF FOODS?

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Foods that contain phytate have for several decades generally been considered sources of iron of low biological availability. Widdowson and McCance in the early 1940's observed lower iron balances in human subjects consuming brown bread than in those consuming white bread (1). Later they showed that consumption of white bread in which Na phytate had been baked decreased serum iron response; they therefore attributed to phytate the effect of brown bread in lowering iron balance (2). There are other reports which show that Na phytate will decrease iron absorption by humans. Moore et al. (3) concluded iron phytate prepared by precipitation of phytate with a ferric salt to be a poorly utilized form of iron. However, a close examination of the literature raises the question of whether the endogenous or native phytate of foods such as wheat, does in fact, have a deleterious effect on absorption of the native food iron. Callender (4) compared brown and white bread, both containing only the endogenous phytate, and found no difference in the absorption of iron from the two kinds of bread. Cook et al. (5) found the absorption ratio of biosynthetically tagged native wheat iron to tagged supplemental iron baked into the same rolls was 1.2, that is, the native wheat iron was absorbed to a greater extent than the added iron. Sharpe et al. (6) found that rolled oats, which contains phytate, decreased the absorption of radioiron no more than did milk, which does not contain phytate.

Considering the above findings and the important contribution of cereal products, particularly of wheat, to the dietary iron intake of the U.S. population (7) we have undertaken in our laboratory a study of the biological availability of the iron in wheat.

The prophylactic maintenance of hemoglobin in the growing rat was the bioassay parameter for biological availability and relative biological values computed by slope ratio. Ferrous ammonium sulphate  $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$  was used as the reference compound with relative biological values (RBV) expressed as percentage of the response to the reference compound assigned the biological value of 100. The diets contained 20% casein and adequate levels of all minerals except iron; thus trace element and amino acid requirements were satisfied.

Table 1 summarizes experiments we conducted to determine the biological availability to the rat of the iron in wheat. The RBV found for the iron in wheat and its milling fractions, bran, germ and shorts, is about 90, an iron source of high biological availability for the rat.

Table 1. Relative Biological Value to the Rat of the Iron in Wheat and its Milling Fractions<sup>1/</sup>

Fe Source	Hard Wheat	Soft Wheat
Whole grain	90 (72-114) <sup>2/</sup>	88 (70-107)
Bran	86 (70-108)	98 (74-125)
Germ	92 (75-113)	91 (69-121)
Shorts	92 (76-111)	

<sup>1/</sup>Relative biological value is percentage based on response to  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O} = 100$ . Each value is the average of two or three bioassays.

<sup>2/</sup>95% confidence limits in parenthesis.

Results of four separate trials using whole wheat bread are shown in Table 2. The bread used was purchased from the grocery shelf. In only one of the four trials was the RBV for whole wheat bread very high. Even including the values for all four trials, the average RBV was only 75, less than expected on the basis of 90 for the whole grain. The average RBV excluding the high value was 62 and the upper 95% confidence limit for the three lower RBV trials does not overlap the average RBV obtained for the whole grain (Table 1).

Table 2. Relative Biological Value of the Iron in Whole Wheat Bread<sup>1/</sup>

Trial values	115	67	53	65
95% confidence limits	(92-143)	(51-83)	(40-65)	(51-79)

<sup>1/</sup>Relative biological value is percentage based on response to  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 = 100$ .

The rat does possess an intestinal phytase (8), and the ability of the rat to utilize the iron of cereals has been explained on the basis of hydrolysis of the phytate. On this argument we would expect the iron in whole wheat bread to be as well utilized as that in the uncooked whole wheat. We have not found this always to be true. A possible explanation for this result would be an alteration of the native wheat iron to a less available chemical form during the baking process. Although we did not measure the phytate content of the flour used to bake the bread, the bread would be expected to have the lower phytate content (9).

We also tested several whole grain or bran cereals with the results given in Table 3. Although two of the cereals were ready to eat varieties, all three underwent some degree of cooking or subjection to heat and probably moist heat. The instant cereal to be served hot had a lower RBV than the whole grain in Table 1, but the ready to eat bran and whole wheat cereals had RBV equal to or greater than the whole grain. Thus, cooking does not always alter the biological availability to the rat of the native iron in wheat products.

Table 3. Relative Biological Value of the Iron in Wheat Cereals

Cereal	RBV <sup>1/</sup>	95% Confidence Limits
Ready to eat, whole wheat	100	81-121
Ready to eat, bran	89	74-107
Instant whole wheat, to be served hot	72	55-70

<sup>1/</sup>Relative biological value is percentage based on response to  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O} = 100$ .

Investigation into the chemical nature of the iron in wheat had been started before all the results from the bioassays cited above were completed. The bioassay of the whole wheat bread and the cereals provided additional evidence that the chemical nature of the native wheat iron might be the factor determining biological availability. There is some cytochrome iron in the germ, but the larger percentage of the iron in wheat is nonheme (10) and ferric in oxidation state (11). Bran was chosen as starting material because it accounts for most of the iron in wheat.

We began our study by a series of extractions by different solvents in this order: butanol, water and aqueous salt outlined in figure 1.

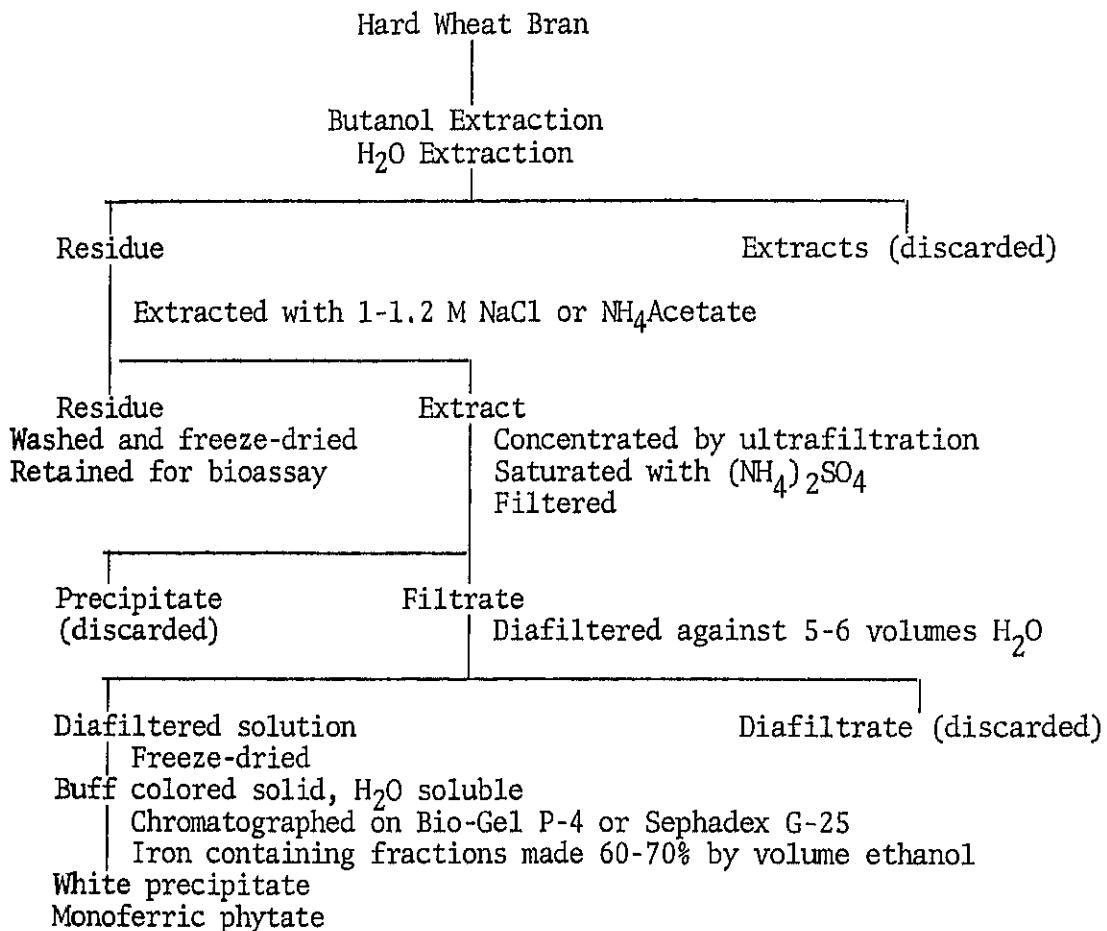


Figure 1. Isolation and purification of monoferric phytate from wheat bran.

Aqueous solvents were at 10 ml per gram of bran. All extractions were carried out in the cold. No iron was found in the butanol and water extracts, but exhaustive extraction by 1 molar NaCl solubilized 60-70% of the iron in bran. We later used NH<sub>4</sub> acetate instead of NaCl and were able to attain the same degree of extractability of the iron. The amount of iron extracted was significantly reduced if salt solutions of less than 1 molar were used or if the salt solution was buffered at pH greater than 7. The gel filtration chromatography pattern of the salt extract is shown in figure 2a. Similar patterns were obtained on either Sephadex G-25 or Bio-Gel P-4. These gels both have nominal exclusion limits of 5,000 daltons. The major iron coincided with fractions that contained phosphorus as well as some material that absorbed u.v. radiation of 280 nm. Early speculation, therefore, was on a low molecular weight phosphopeptide.

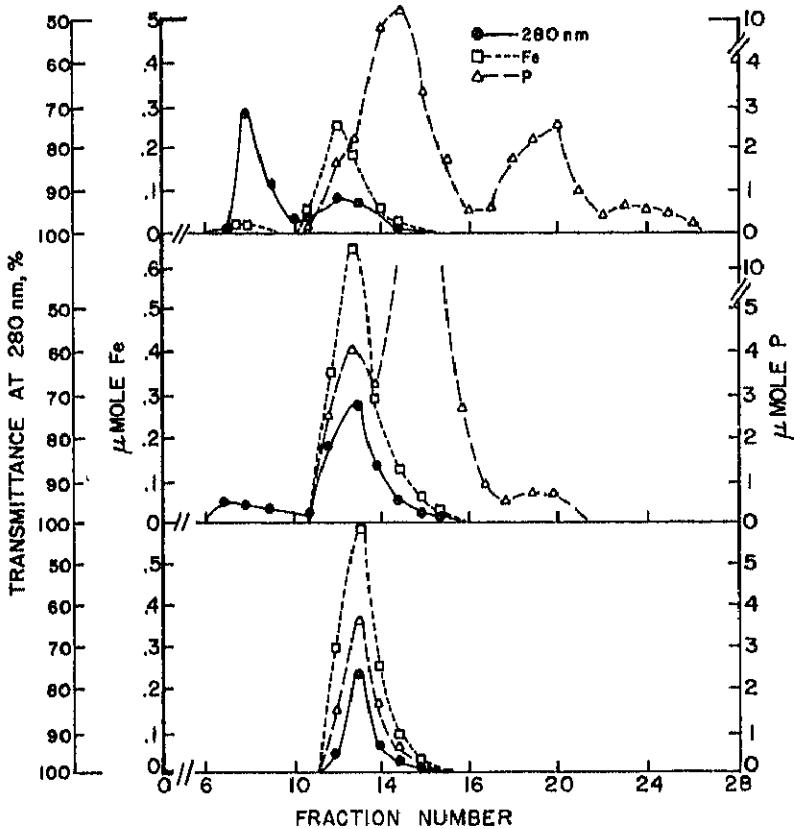


Figure 2. Gel Chromatography Patterns:

- (Upper) Concentrated salt extract of wheat bran.
- (Middle) Diafiltered solution or freeze-dried product.
- (Lower) Monoferric phytate. Chromatographed on Bio-Gel P-4 using 1.2 M  $\text{NH}_4$ acetate buffer.

The salt extract was then concentrated, the ammonium sulfate precipitable protein removed and the ammonium sulfate and salt removed by diafiltration. The iron complex remained in solution. The gel chromatographic pattern of the diafiltered solution is shown in figure 2b. Most of the higher weight material which absorbed 280 nm radiation was now removed as well as the lower molecular weight P. The iron fractions still coincided with a 280 nm absorption peak, and although some P was found in the iron fractions, most of the P was in a separate peak. We freeze dried this diafiltered solution and obtained a buff colored solid which was readily water soluble. Most preparations contained about 1,000 ppm Fe and 60,000 ppm P. The major P peak of this freeze dried product was determined to be phytate, and the iron fractions contained P to Fe approaching an 8:1 molar ratio. Final separation of the iron compound was attained by addition of ethanol to the iron containing gel chromatography fractions and collection of the

resultant white precipitate. When this white precipitate was redissolved in NH<sub>4</sub>acetate and chromatographed on the gel column, the chromatogram showed a single peak of P and Fe that coincided with absorption of 280 nm radiation, figure 2c. The iron compound was determined to be monoferric phytate by P, Fe and inositol analysis. The gel chromatographic pattern and u.v. absorption spectrum (figure 3) was identical to that produced by synthetic monoferric phytate produced from Na phytate and ferric chloride.

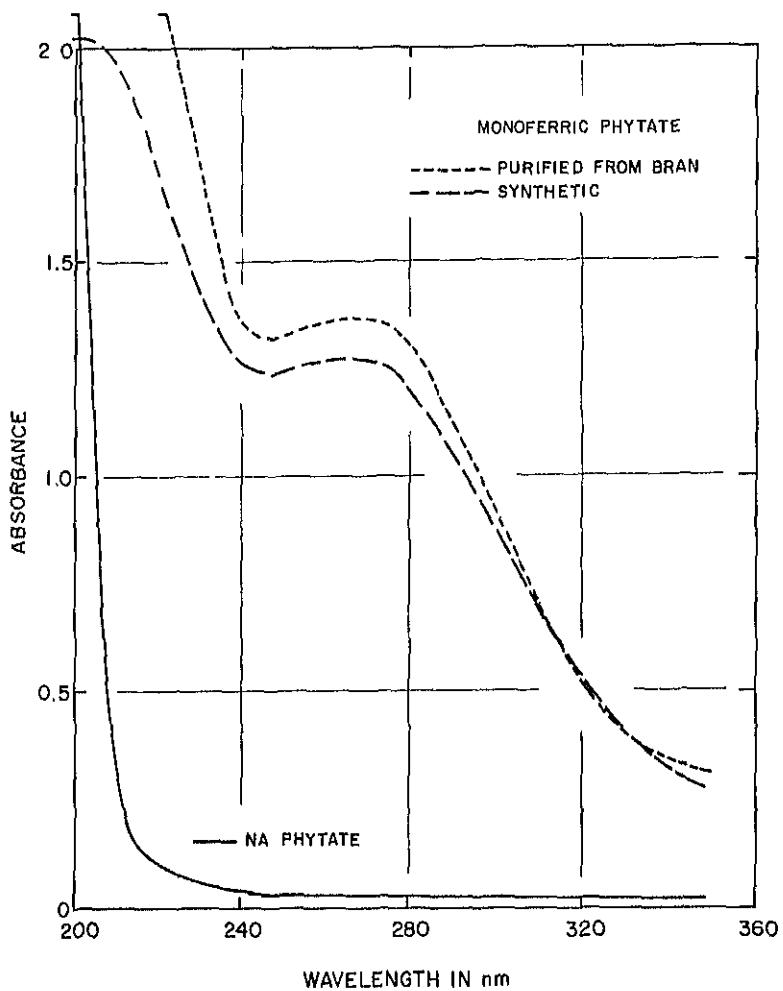


Figure 3. Ultraviolet Absorption Spectra.

Results of hemoglobin depletion-repletion biological availability assay are shown in figure 4. Included in this bioassay was the freeze dried product isolated from wheat bran (Fe phytate-bran), two synthetic monoferric phytate preparations (Fe phytate 1&2), and the water and salt extracted bran residue, a synthetic monoferric phytate plus ascorbic acid and a single level of a saturated ferric phytate preparation (Fe<sub>4</sub>phytate).

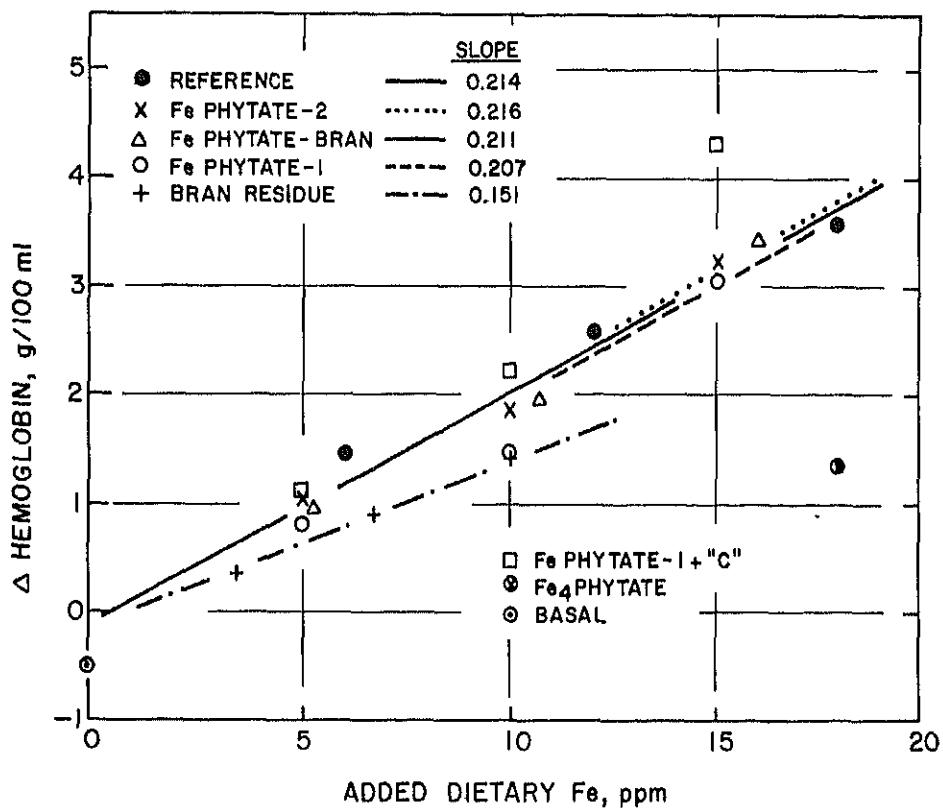


Figure 4. Slope ratio analysis of biological availability assay.

Fe phytate-1 was prepared by adding ferric chloride to a solution of Na phytate (all in 0.5M acetic acid) at a 1:1 molar ratio and freeze drying.

Fe phytate-2 was prepared similarly except that the product was precipitated by addition of ethanol to 60% by volume. Although labeled in figure 4 as Fe<sub>4</sub>phytate the actual analysis of the saturated ferric phytate was 3.2 moles Fe/phytate. The relative biological values were (reference compound Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>.6H<sub>2</sub>O=100) Fe phytate-2, 102; Fe phytate-bran, 99; Fe phytate-1, 97 and bran residue 71. The three monoferric phytate preparations were equivalent in biological availability and also to the reference compound. The bran residue, on the other hand, was only .7 as efficient as the reference compound and the calculated 95% confidence limits indicate significantly lower biological availability of the iron. The bran residue contained no detectable phytate. The chemical form of the iron in the bran residue is different from and of lower biological value than monoferric phytate. Reinhold (12) recently showed that dephytinized bran will bind ferric iron. No RBV was calculated for Fe phytate-1 + ascorbic acid because of significant departure from linearity. Only at the highest level of added iron was the response with ascorbic acid greater than without; this indicated that ascorbic acid does not significantly improve the utilization of monoferric phytate. This is in agreement with the previous finding in our laboratory that 1% ascorbic acid did not significantly improve the hemoglobin response to the iron of whole wheat. The response to the single level of saturated ferric phytate also confirms other observations in our laboratory that this is an iron source of poor biological availability to the rat.

The basal diet we used in the bioassays was 20% vitamin free casein, 4% low iron salts, 1% vitamin mix, 5% corn oil and 70% dextrose. A recent report by Amine and Hegsted showed that the absorption of inorganic iron by the rat was lowest when starch was used as the carbohydrate in purified diets (13). We recently used starch in place of the dextrose in a bioassay and found no reduction in RBV of monoferric phytate. Tests are currently underway to answer the question of whether baking might alter the chemical form of monoferric phytate added in bread, and whether such alteration might consequently change the biological availability.

Table 4 is a summary of a balance study designed to ascertain the ability of the rat to either hydrolyze or otherwise utilize phytate. Diets of the same level of iron with four different sources of phytate were fed to 100-g rats for 1 week, followed by a 10-day balance trial. Feces were dried in vacuo and ground in a micro Wiley mill before analysis.

Table 4. Phytate and Iron Balance of Rats Fed Different Types of Phytate<sup>1/</sup>

Fe Source	Phytate Source	% Excreted in Feces		Final Hemoglobin
		Phytate	Iron	
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O	None	-	68	14.5
Monoferric phytate	Monoferric phytate	3.5	64	12.4
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O	Na phytate	0.8	64	12.0
Bran	Bran	46	65	13.3

<sup>1/</sup>Diets contained 29-32 ppm Total Fe. Phytate contents were 0.0, 0.02, 0.02 and 0.74%.

Preliminary to analysis of the feces for phytate, it was found that acid extraction and precipitation by ferric iron resulted in overestimation of phytate in materials with high inorganic phosphate content. A short incubation in 0.5 M HCl was found to eliminate the interference by phosphate. The feces in this experiment were analyzed with a 2-hour incubation of the precipitate in 0.5 M HCl. No phytate was detected in the feces when the diet contained no phytate. Practically no phytate was excreted in the feces when the diet contained 0.02% phytate as either monoferric phytate or Na phytate with iron as ferrous ammonium sulfate, and slightly less than one-half of the phytate was excreted when bran was added to provide 0.7% phytate in the diet. Approximately 35% of the iron was absorbed by animals regardless of which of the four diets they were fed. Thus it cannot be said with certainty that enzymatic hydrolysis of phytate and thus release of iron does not account for the efficient utilization of iron in cereals by the rat. If enzymatic hydrolysis of monoferric phytate does occur, what are the possible end products? Ferric phosphate is one possibility, but this has a low biological availability. Release of the iron as ferric iron would almost certainly result in precipitation as ferric hydroxide except in the very proximal portion of the small intestine. We have made ferric hydroxide and bioassayed its availability with the resultant regression line having almost 0 slope. It is possible that monoferric phytate may be absorbed intact.

The solubility characteristics of monoferric phytate seem more likely to be the explanation for its high biological availability. We have not determined its maximum solubility, but have dissolved at least 20 mg/ml in either deionized water or NH<sub>4</sub>acetate. About 80% of the amount applied to a gel column in 0.05 M buffer was recovered in the eluate over a pH range of 4.5-10. Although our initial extracts were made with aqueous molar NaCl, recovery was decreased when 0.5 M NaCl was included in the gel chromatography experiments over the same pH range. Lower pH does decrease the solubility of monoferric phytate, particularly if it is heated. Larger aggregates or polymers apparently do form if the ionic strength is 0.1 or less for the gel chromatography experiments, but at the ionic strength of the GI tract, aggregation is not likely.

One asks the question "is monoferric phytate formed as an artifact of the extraction of the wheat bran?" We do not believe this to be true. Approximately one-half of the phytate of bran was found to be water soluble, yet no iron was extracted by water. Within minutes after addition of NH<sub>4</sub>acetate solution the extract contained iron that chromatographed in the gel column as monoferric phytate. Monoferric phytate in water was found to be bound by the water and NH<sub>4</sub>acetate extracted bran residue and could be reextracted only upon the addition of NH<sub>4</sub>acetate solution. The bran seems almost equivalent to a strong anion exchange resin in binding affinity for monoferric phytate.

We plan to test the absorption of monoferric phytate by humans. The solubility and stability characteristics of monoferric phytate indicate that it may be absorbed very well in contrast to the insoluble saturated ferric phytate. Rather than the phytate effect, the presence of food or fiber may just as well be the explanation for low absorption of iron in cereals by humans. Phytase activity has been demonstrated in human intestine (14) and, although this activity may be only a phosphatase activity and although its quantitative aspects are uncertain, we cannot ascribe the difference between the ability of the human and rat to utilize the iron in cereals simply to the presence of an intestinal phytase only in the rat. Furthermore, Welch and Van Campen have shown that the rat utilized the iron of mature soybeans high in phytate to a greater extent than of immature soybeans low in phytate (15).

Conclusion: Monoferric phytate has been isolated from wheat bran and accounts for about one-half the iron in whole wheat. Biological availability to the rat of iron as monoferric phytate purified from bran or produced synthetically from Na phytate and ferric chloride is equivalent to ferrous ammonium sulfate. In contrast to saturated ferric phytate, monoferric phytate is soluble and reasonably stable over the pH range expected in the gastrointestinal tract. Its stability in foods such as bread is being investigated.

### References

1. Widdowson, E. M. and R. A. McCance (1942) Iron exchanges of adults on white and brown bread diets. *Lancet* i: 588-591.
2. McCance, R. A., C. H. Edgecombe and E. M. Widdowson (1943) Phytic acid and iron absorption. *Lancet* ii: 126-128.
3. Moore, C. V., V. Minnich and R. Dubach (1943) Absorption and therapeutic efficacy of iron phytate. *J. Amer. Diet. Assoc.* 19: 841-844.
4. Callender, S. T. and G. T. Warner (1970) Iron absorption from brown bread. *Lancet* i: 546-547.
5. Cook, J. D., V. Minnich, C. V. Moore, A. Rasmussen, W. B. Bradley and C. A. Finch. Absorption of fortification iron in bread. *Amer. J. Clin. Nutr.* 26: 861-872.
6. Sharpe, L. M., W. C. Peacock, R. Cooke and R. S. Harris (1950) The effect of phytate and other food factors on iron absorption. *J. Nutr.* 41: 433-446.
7. Morris, E. R. (1974) Nutritional significance of trace elements in wheat. In Proc. 8th National Conference on Wheat Utilization Research, D. A. Fellers, ed.
8. Pileggi, V. J. (1959) Distribution of phytase in the rat. *Arch. Biochem. Biophys.* 80: 1-8.
9. Reinhold, J. G. (1972) Phytate concentrations of leavened and unleavened Iranian breads. *Ecol. Food Nutr.* 1: 187-197.
10. Cook, J. D., Unpublished results.
11. Morris, E. R., F. E. Greene and A. C. Marsh. On the chemistry and biological availability to the rat of the iron in wheat. In Trace Element Metabolism in Animals-2, W. G. Hoekstra et al. ed., University Park Press, Baltimore, 1974 p. 506-508.
12. Reinhold, J. G., F. Ismail-Beigi and B. Faradji (1975) Fibre vs. phytate as determinant of the availability of calcium, zinc and iron of breadstuffs. *Nutr. Rpts. Int.* 12: 75-85.

13. Amine, E. K. and D. M. Hegsted (1975) Effect of carbohydrates and fats on inorganic iron absorption. *J. Agr. Food Chem.* 23: 204-208.
14. Bitar, K. and J. G. Reinhold (1972) Phytase and alkaline phosphatase activities in intestinal mucosae of rat, chicken, calf and man. *Biochim. Biophys. Acta* 268: 442-452.
15. Welch, R. M and D. R. Van Campen (1975) Iron availability to rats from soybeans. *J. Nutr.* 105: 253-256.

## WHEAT PROTEINS, AMINO ACID SEQUENCING, AND CELIAC DISEASE

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Introduction. Celiac disease, also known by other names such as gluten-sensitive enteropathy, celiac sprue, and non-tropical sprue, is a relatively rare condition in which the susceptible individual must totally exclude wheat or products containing wheat proteins from the diet. The incidence in the United States is generally assumed to be about the same as that in Western Europe where it affects roughly 1 in 2000 although the incidence does show some geographical variation and has been estimated to be as high as 1 in 300 in the West of Ireland (1).

The disease is genetically determined by more than one gene (2), but environmental factors seem to play a role in the onset of symptoms as evidenced by the appearance of the disease in only one of a set of identical twins (3). The toxic effects of wheat in the diet of an individual with celiac disease are localized in the small intestine. This is the site where almost all nutrients are absorbed -- amino acids, sugars, vitamins, etc. The inner surface or epithelium of the small intestine is covered with small, finger-like projections called villi that serve to extend the absorptive surface area. Small depressions or holes called crypts extend beneath the surface. The entire surface is covered with a single layer of cells that are formed at the base of the crypts and migrate up the walls of the crypts to the surface and then up the walls of the villi. They are eventually sloughed off from the tips of the villi into the interior of the small intestine. The entire population of epithelial cells is continually renewed by this process every few days. The cells undergo a maturation process during this migration whereby they increase their enzymatic activities and absorptive capacities (4).

The surface of the epithelial cells that cover the villi have, in turn, tiny, fingerlike projections called microvilli. Whereas the villi are about a millimeter in length, the microvilli are only about a micron in length. Many of the enzymes found in the absorptive cells are associated with the plasma membrane covering the microvilli. These enzymes include disaccharidases, peptidases, alkaline phosphatase, and ATPase among others.

'When an individual with celiac disease eats wheat, adverse changes in the epithelial surface of the small intestine result; these changes have been studied by examination of small bits of tissue obtained from the intestine by a biopsy procedure whereby a small capsule attached to flexible tubing is inserted into the intestine by way of the esophagus. The villi become shortened apparently because of tissue destruction while the crypts become thickened as a consequence of enhanced cell proliferation. The net result is a flattening of the mucosal surface and a considerable decrease in absorptive capacity probably due to a decrease in surface area and to a loss of mature absorptive cells.

Immediate symptoms of celiac disease may include diarrhea and general intestinal distress. Malabsorption of fats usually occurs and results in pale, bulky stools. Failure to digest and absorb disaccharides usually results in diarrhea by reversing the osmotic flow of water in the large intestine. Bacterial fermentation of undigested foodstuffs in the colon may also contribute compounds that cause irritation and produce diarrhea. Eventually, any symptoms that can result from malabsorption of nutrients may occur. A loss of weight in adults and a failure to grow in children frequently accompany celiac disease.

Role of Wheat Proteins. Although the symptoms of celiac disease have been recognized since antiquity, it was not until 1950 that Dicke demonstrated that wheat was responsible and that the toxic factor was associated with the proteins rather than with the starch (see discussion of ref. 5). Rye also produced the symptoms as does barley. Until recently, it was thought that oats were toxic as well, but it now seems that oats may be eaten safely by celiac patients (6). Corn and rice do not produce the symptoms and these cereals also may be eaten safely by celiac patients, but unfortunately they do not produce a satisfactory bread. Since it is necessary for the celiac patient to exclude all foods from the diet that might contain wheat proteins, many patients find adherence to the required diet a considerable hardship. As long as wheat is totally excluded from the diet, however, most patients remain free of symptoms. Removal of wheat from the diet of a patient with active disease usually results in rapid recovery although return of the intestinal mucosa to a nearly normal state may take months to years.

The gliadin proteins were found mainly responsible for the toxic effects of wheat proteins although some toxicity was also shown by glutenin preparations (7). Combined digestion with pepsin and trypsin was not effective in destroying the toxicity of gliadin proteins (8). Evidently, peptides derived from gliadin (and perhaps glutenin) proteins during the digestive process are responsible for the toxic effects. Intact proteins may be toxic as well (9).

The mechanism by which gliadin proteins produce the symptoms of celiac disease is not known. It has been suggested that susceptible individuals lack an enzyme that, in normal individuals, breaks down some toxic peptide (10) derived from gliadins. So far, however, no enzyme defect has been demonstrated in celiac patients although almost all the enzymatic activities associated with the epithelium of the small intestine are depressed when the disease is active.

Immune responses localized in the small intestine clearly are involved (11) and are apparently responsible for the tissue damage. The immune response might be triggered by some toxic peptide, but it is also possible that an abnormal immune response is triggered by a peptide that is not toxic to the normal person. This latter possibility seems more likely insofar as celiac patients have an unusually high incidence of a particular histocompatibility antigen (cell surface protein) called HL-A8 (12).

Toxicity of A-gliadin. The gliadin proteins are a mixture of a considerable number of different protein components. These components have similar amino acid compositions, but they do differ significantly and can be

separated from one another by gel electrophoresis and other physical methods. Wrigley and Shepherd (13) used a combination of gel electrophoresis and iso-electric focusing to demonstrate that a gliadin preparation derived from a single wheat variety contained more than 40 protein components. There are differences in protein components among different wheat varieties and, consequently, there may be hundreds of distinguishable gliadin protein components. The distribution of the amino acid sequence constituting the smallest peptide capable of producing the toxic effects has not been determined with respect to the many gliadin components.

The toxicity of one particular gliadin fraction, originally separated in our laboratory (14) and which we now call A-gliadin (15), seems well established, however. Hekkens *et al.* (9) instilled A-gliadin directly into the small intestine of patients with celiac disease in remission (on a wheat-free diet); changes in the epithelial tissue that were characteristic of celiac disease occurred within hours. The toxicity of A-gliadin was also demonstrated by Falchuk *et al.* (4) by means of an organ culture test. When a small piece of tissue obtained from the intestine of celiac patients with active celiac disease was cultured in the presence of A-gliadin, the development of certain enzyme activities was repressed. This repression was not found for tissues obtained from normal persons or for tissues obtained from celiac patients with the disease in remission.

Amino Acid Sequencing. Since we had carried out fairly extensive characterization of A-gliadin in our laboratory, largely because we thought it served as a useful model for studying protein interactions in doughs, we decided to carry out amino acid sequencing studies of this fraction in the hope of identifying the toxic sequence. We have been collaborating with Dr. Warren Strober's group at the National Institutes of Health by supplying them with A-gliadin and peptides derived from A-gliadin for testing with the organ culture test devised by them. The advantage of this test lies in the requirement for milligram amounts of material as opposed to the gram amounts of material needed for tests that involve patients directly. At the same time, we have been attempting to determine the amino acid sequences of the peptides that we have obtained from the intact protein by several different chemical and enzymatic methods. Our ultimate goal will be to determine the minimal amino acid sequence capable of producing toxic effects and its distribution in the protein molecule. We would also hope to determine the distribution of this sequence in other gliadin proteins as well.

Although our work is in progress, the NIH workers have obtained sufficient amounts of data on two of our peptides (16) to indicate that both are toxic to organ cultures derived from patients with active disease. The amino acid compositions and partial amino acid sequences that we have obtained for these peptides are given below:

PEPTIDE 30.21  
Molecular Weight ~6,600

Composition

Glx<sub>21</sub>Leu<sub>7</sub>Pro<sub>6</sub>Val<sub>3</sub>Ala<sub>3</sub>Ser<sub>3</sub>Thr<sub>2</sub>Arg<sub>2</sub>Asx<sub>2</sub>Gly<sub>2</sub>Ile<sub>2</sub>Phe<sub>2</sub>Tyr<sub>1</sub>His<sub>1</sub>Cys<sub>1</sub>Met<sub>1</sub>

Sequence

1               5               10               15  
-Asp-Val-Val-Leu-Gln-Gln-<sup>Val</sup>-Asn-Leu-Ala- ? -Gly- ? -Gln-Gln-  
16               20               25  
Val-Leu-Leu-Gln-Gln-Tyr-Tyr-Leu-Leu-.....Met.

PEPTIDE 30,38  
Molecular Weight ~4,000

Composition

Pro<sub>5</sub>Ile<sub>5</sub>Tyr<sub>3</sub>Phe<sub>3</sub>Gly<sub>3</sub>Asx<sub>3</sub>Cys<sub>3</sub>Thr<sub>3</sub>Val<sub>2</sub>Ala<sub>2</sub>Met<sub>1</sub>

Sequence

1               5               10               15  
-Cys-Asn-Val-Tyr-Ile-Pro-Pro-Tyr- ? - ? -Ile-Ala-Pro-Phe-Gly  
16  
Ile-Phe-Gly-Pro-.....Met.

Because the compositions of these two peptides differ considerably, we have been puzzled by the finding that both are toxic. We cannot rule out yet the possibility that they have some region of sequence in common and we are also considering the possibility that one of the preparations might contain minor amounts of the other as a contaminant. Upon resolving this question, we shall proceed to break down the toxic peptide and attempt to determine the smallest possible toxic peptide -- and its amino acid sequence.

Possibility of Obtaining a Non-toxic Wheat. Kendall and coworkers (17) separated a gliadin mixture into a number of fractions by using ion-exchange chromatography on carboxymethyl cellulose. They fed these fractions to celiac patients in remission and noted the effects on the ability of the patients to absorb xylose; a drop in xylose absorption indicated that the protein fraction was toxic. On the basis of the xylose absorption test, they concluded that only one of their protein fractions was toxic and they noted that the mobilities of the components in this fraction mainly corresponded to those of alpha-gliadins upon gel electrophoresis. If it should turn out that toxicity of gliadin proteins is limited to a few components, it may be possible to obtain a genetic variant of hexaploid wheats that does not contain these toxic proteins.

For example, the A-gliadins (which are toxic) are controlled by the 6A chromosome in hexaploid wheats (18). A genetic variant missing that chromosome has been prepared by Sears (19). We are planning to investigate the possibility that this genetic variant, or some other variant, might be eaten safely by celiac patients; these studies are being carried out in conjunction with Prof. C. O. Qualset of the University of California, Davis, and with Dr. Strober's group at the National Institutes of Health.

It may turn out that the toxic sequence is widely distributed among the many gliadin protein components. Indeed, there is evidence that many of these protein components share common sequences (although not necessarily the toxic sequence) (20, 21). Wide distribution of the toxic sequence would make it impossible to obtain a non-toxic wheat by genetic means.

#### Acknowledgment

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#### Literature Cited

1. Mylotte, M., Egan-Mitchell, B., McCarthy, C. F., and McNicholl, B. Incidence of coeliac disease in the West of Ireland. *Br. Med. J.* 1: 703, (1973).
2. McCrae, W. M. The inheritance of coeliac disease. In *Coeliac Disease*. C. C. Booth and R. H. Dowling (Eds.). *Proc. Int. Symposium*. Churchill Livingstone, Edinburgh, p. 55 (1970).
3. Walker-Smith, J. A. Discordance for childhood coeliac disease in monozygotic twins. *Gut*. 14: 374 (1973).
4. Falchuk, Z. M., Gebhard, R. L., Sessions, C., and Strober, W. An in vitro model of gluten-sensitive enteropathy: Effect of gliadin on intestinal epithelial cells of patients with gluten-sensitive enteropathy in organ culture. *J. Clin. Invest.* 53: 487 (1974).
5. Douglas, A. P. Long term prognosis and relation to diets. In *Coeliac Disease*. W. Th. J. M. Hekkens and A. S. Pena (Eds.). *Proc. 2nd Int. Symp.* Stenfert Kroese, Leiden, p. 399 (1974).
6. Dissanayake, A. S., Truelove, S. C., and Whitehead, R. Lack of harmful effects of oats on small intestinal mucosa in coeliac disease. *Brit. Med. J.* 4: 189 (1974).
7. Van de Kamer, J. H., Weijers, H. A., and Dicke, W. K. Coeliac disease. IV. An investigation into the injurious constituents of wheat in connection with their action on patients with coeliac disease. *Acta Paediat.* 42: 223 (1953).
8. Frazer, A. C. On the significance of mucosal damage. In *Intestinal Biopsy*. G. E. W. Wolstenholme and M. P. Cameron (Eds.). Ciba Foundation Study Group No. 14. Little, Brown and Co., Boston, p. 54 (1962).
9. Hekkens, W. Th. J. M., Haex, A. J. Ch., and Willighagen, R. G. J. Some aspects of gliadin fractionation and testing by a histochemical method. In *Coeliac Disease*. C. C. Booth and R. H. Dowling (Eds.). *Proc. Int. Symp.* Churchill Livingstone, Edinburgh, p. 11 (1970).

10. Frazer, A. C. Discussion on some problems of steatorrhea and reduced stature. *Proc. Roy. Soc. Med.* 49: 1009 (1956).
11. Shiner, M. Cell distribution in the jejunal mucosa in coeliac disease. In *Coeliac Disease*. W. Th. J. M. Hekkens and A. S. Pena (Eds.). *Proc. 2nd Int. Symp.* Stenfert Kroese, Leiden, p. 121 (1974).
12. Falchuk, Z. M., Rogentine, G. N., and Strober, W. Predominance of histo-compatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. *J. Clin. Invest.* 51: 160 (1972).
13. Wrigley, C. W., and Shepherd, K. W. Electrophoresis of grain proteins from wheat genotypes. *Annals N. Y. Acad. Sci.* 209: 154 (1973).
14. Bernardin, J. E., Kasarda, D. D., and Mecham, D. K. Preparation and characterization of alpha-gliadin. *J. Biol. Chem.* 242: 445 (1967).
15. Platt, S. G., Kasarda, D. D., and Qualset, C. O. Varietal relationships of the  $\alpha$ -gliadin proteins in wheat. *J. Sci. Food Agric.* 25: 1555 (1974).
16. Kasarda, D. D., Nimmo, C. C., and Bernardin, J. E. Structural aspects and genetic relationships of gliadins. In *Coeliac Disease*. W. Th. J. M. Hekkens and A. S. Pena (Eds.). *Proc. 2nd Int. Symp.* Stenfert Kroese, Leiden, p. 25 (1974).
17. Kendall, M. J., Schneider, R., Cox, P. S., and Hawkins, C. F. Gluten subfractions in coeliac disease. *Lancet.* II: 1065 (1972).
18. Kasarda, D. D., Bernardin, J. E., and Qualset, C. O. Relationship of gliadin protein components to chromosomes through the use of substitution lines. *Cereal Sci. Today* 9: 403 (1974).
19. Sears, E. R. The aneuploids of common wheat. *Missouri Agr. Exp. Sta. Res. Bull.* 572, 59 p. (1954).
20. Bietz, J. A., Huebner, F. R., and Rothfus, J. A. Chromatographic comparisons of individual gliadin proteins. *Cereal Chem.* 47: 393 (1970).
21. Ewart, J. A. D. Fingerprinting of glutenin and gliadin. *J. Sci. Food Agric.* 17: 30 (1966).

# STATUS OF CHEMICAL METHODS TO DETERMINE BIOLOGICALLY AVAILABLE LYSINE IN WHEAT PROTEINS

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We have all heard the term "available lysine" quite often and likewise, we probably all understand that "available" in this context refers to nutritionally available forms of the amino acid lysine; that is, lysine which may be used for anabolic physiological purposes such as protein synthesis.

Nevertheless, I would like to initiate this discussion with a very brief review of possible causes of losses in nutritional availability of lysine. Following this, I will briefly describe the currently available chemical approaches or methods for estimating how much of the lysine in any food protein is available and then focus on the applications of these methods to the analyses of high-carbohydrate food protein sources such as wheat or products derived from wheat. Only limited information exists about the relationship between various chemical estimates of available lysine and actual nutritional value for animals and humans. Some questions will be posed on the usefulness of such estimates of available lysine in high-carbohydrate foods and the possible lack of significance, in human nutrition, of chemical estimates of available lysine in wheat products.

"Available" Lysine. The structure of the amino acid, lysine, in its free form (i.e., with a free alpha amino group, a free epsilon-amino group and a free carboxyl group) is shown in Figure 1(a). Ninety-five to 100% of the lysine in most proteins is linked to other amino acids through peptide linkages involving the alpha-amino and carboxyl groups [Fig. 1(b)]. Only the epsilon amino group is thus free to react with various substances such as sugars or amide groups of other amino acids. The probability of such reactions occurring is increased by heating.

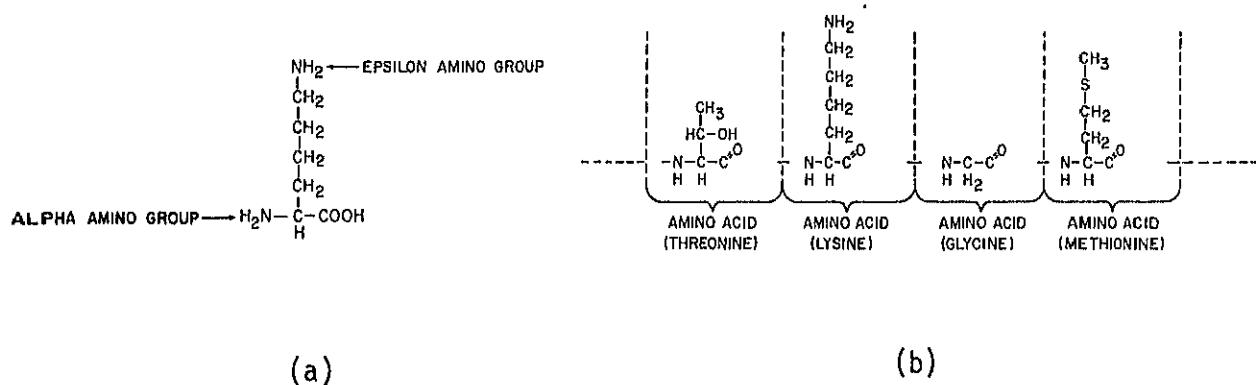


Figure 1. (a) Structure of free lysine showing free epsilon and alpha amino groups; (b) lysine as it would exist in a portion of a peptide chain of a theoretical protein; note free epsilon amino group.

If the epsilon amino group is "tied up" through a chemical reaction, the segment of the protein near the affected lysine residue may not be digested (the proteolytic enzyme, trypsin, acts on a protein at either an arginine residue or a lysine residue which has a free amino group), or, if it is broken down to the amino acid or dipeptide level, the reacted lysine may not be absorbed from the gastrointestinal tract. Even if absorbed, the lysine having a "derivatized" epsilon amino group may not be utilized but rather may be excreted in the urine.

In theory then, a lysine residue which has a free epsilon amino group should be nutritionally available; likewise, lysine in which the epsilon amino group is not free may be nutritionally unavailable (10,33).

Chemical Methods for Estimating Available Lysine. Since the first application of Sanger's technique (45) to foods by Carpenter and others (9,11,12), all chemical methods for estimating the available lysine in a protein or food have been simply methods intended to estimate, either directly or indirectly, how much of the lysine is present which has a free unreacted epsilon amino group [see Carpenter and Booth (10) for an excellent review]. The estimated amount of free lysine expressed as a percentage of the total lysine present results in a value for the percent available lysine. Total lysine, as estimated by amino acid analysis, includes both available and unavailable lysine since the reacted epsilon amino group in the nutritionally unavailable lysine is usually converted to the free "unreacted" form upon acid hydrolysis.

The general approach for chemically estimating nutritionally available lysine can be summarized: (a) a chemical agent is reacted with a protein and an acid stable derivative is formed between free epsilon amino groups of lysine in the protein and the chemical agent; (b) the "derivatized" protein is hydrolyzed to yield derivatized lysine plus other free amino acids (including non-derivatized, unreacted, i.e., "unavailable", lysine); and (c) the amount of derivatized lysine is determined (direct methods) and/or the amount of unreacted lysine is determined (indirect methods).

Direct Methods. The direct methods currently in use or proposed are listed in Table 1, together with the reagents used, and the derivatives formed and determined by analyses. Of the reagents listed, the long reaction times (up to 48 hours) for the approach using methylisourea has precluded any extensive use of the approach, and only a limited number of applications have been reported (17,34). The use of this reagent will not be discussed further. A recently proposed method (23,42), which utilizes <sup>19</sup>F NMR techniques to quantitate lysine epsilon amino groups reacting with S-ethyl trifluoroacetate, has also not been applied to any extent and will likewise not be discussed further. [The use of dye-binding techniques is not discussed. With high-carbohydrate protein sources, with the exception of very specific applications, a useful relationship between dye-binding capacity and available lysine levels is not readily apparent (30,35)].

The procedures used for the FDNB (1-fluoro-2,4-dinitrobenzene) and TNBS (2,4,6-trinitrobenzene sulfonic acid) direct methods are similar. As an example of these procedures, a schematic representation of the FDNB direct

TABLE 1. Reagents used and derivatives formed and measured in direct methods

Method	Reagent Used	Derivative Formed	Determined by Analyses
"FDNB" OR "CARPENTER" (9, 11, 12, 13)	FDNB (1-fluoro-2,4-dinitrobenzene)	DNP-Lysine ( $\epsilon$ ,N-dinitrophenyl-lysine)	DNP-Lysine
"TNBS" (27)	TNBS (2,4,6-trinitrobenzene sulfonic acid)	TNP-Lysine ( $\epsilon$ ,N-trinitrophenyl-lysine)	TNP-Lysine
Guanidination (34)	O-Methylisourea	Homoarginine	Homoarginine
$^{19}\text{F}$ NMR (42)	S-Ethyl trifluoroacetate	$\epsilon$ ,N-trifluoroacetyl-lysine	$\epsilon$ ,N-trifluoroacetyl-lysine

TABLE 2. Reagents used and derivatives formed in indirect methods

Method	Reagent	Derivative Formed	Determined by Analyses
Total Lysine Minus Inaccessible Lysine (4,40,50)	FDNB	DNP-Lysine	Inaccessible Lysine (unreacted lysine)
Total Lysine Minus Inaccessible Lysine (41)	TNBS	TNP-Lysine	"
Total Lysine Minus Inaccessible Lysine (16)	Methyl Acrylate	$\epsilon$ , $\epsilon$ ,N,N-dicarboxyethyl-lysine and $\epsilon$ ,N-monocarboxyethyl lysine	"
Total Lysine Minus Inaccessible Lysine (18)	Ethyl Vinyl sulfone	$\epsilon$ ,N-(ethylsulfonylethyl) lysine or $\epsilon$ , $\epsilon$ ,N,N-bis-(ethylsulfonylethyl) lysine	"

method is presented in Figure 2. In the direct method, the amount of derivative formed is measured and expressed as lysine equivalents. The total lysine (including available and unavailable lysine) is determined by column chromatography on an acid hydrolysate. The amount of derivative, as lysine equivalents, is then expressed as the percent available lysine.

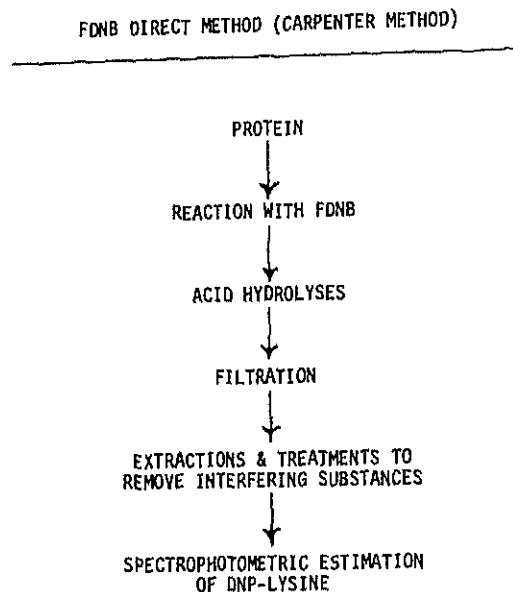


Figure 2. Schematic representation of procedure for estimating available lysine by the FDNB-direct method.

As will be more critically discussed later, for many animal proteins, use of the FDNB direct method appears to result in estimates of available lysine which are generally correlated with the results of animal bioassays. However, with high carbohydrate foods or protein sources serious analytical problems exist. Non-specific side reactions occur which may cause varying degrees of color development which is not related to the amount of available lysine present (see refs. 4,6,15,44). During acid hydrolyses, DNP-lysine (*c,N*-dinitrophenyl-lysine) is partially hydrolyzed. The amount of such hydrolysis may vary from protein to protein. Furthermore, if synthesized DNP-lysine is added as an internal standard, it, too, is hydrolyzed to varying extents depending upon the type of protein being analyzed. Booth (7) has suggested that DNP-protein standards be added and that the percent recovery of the added DNP-protein standard be used to correct for hydrolytic losses in the protein being analyzed. The added standard would be a DNP-derivatized protein similar to the protein being analyzed (e.g., DNP-gluten was added by Booth as an internal standard when analyzing wheat). This procedure is not completely adequate since it involves the accurate determination of the DNP-lysine in the standard protein. Furthermore, the values for wheat which were "corrected"

using this procedure were then comparable to estimates obtained by an indirect procedure (e.g., see Table 4). No advantage is apparent in the use of such correction procedures if the end result is merely the duplication of results obtained by a method which does not involve the use of such correction procedures. During filtering of the acid hydrolyzate, further losses of DNP-lysine occur through the interaction of DNP-lysine with humin (which is formed when carbohydrates are acid hydrolyzed). Filtering while the hydrolyzate is hot appears to reduce the extent of these losses (7). Although many changes in the original procedure have been made or proposed by Carpenter and several others (4,7,9,25,31,32,36,40,43,44,47,48,49), these problems still exist and appear to preclude the use of the FDNB direct method for estimating available lysine in high carbohydrate foods.

Similar problems would appear to exist when TNBS is used in a direct method. In studies of milk proteins, Holsinger and co-workers (23,24) observed that interaction of TNBS with glucosamines and galactosamines resulted in erroneous estimates. Although losses occur during the filtration step, corrections for these losses can be made (41). Both mono- and di-substituted TNP derivatives can be formed and this is also a source of potential error. Variable losses of TNP-lysine during acid hydrolyses undoubtedly occur but this has not been investigated in a detailed study.

Indirect Methods. A schematic representation of the FDNB indirect method for estimating available lysine is presented in Figure 3. The total lysine minus the inaccessible lysine is equivalent to available lysine which can be expressed as a percentage of the total lysine. All of the indirect methods use a similar approach but several different chemical reagents have been used; these are listed in Table 2. In each case, the inaccessible or unreacted lysine (unavailable lysine) is determined.

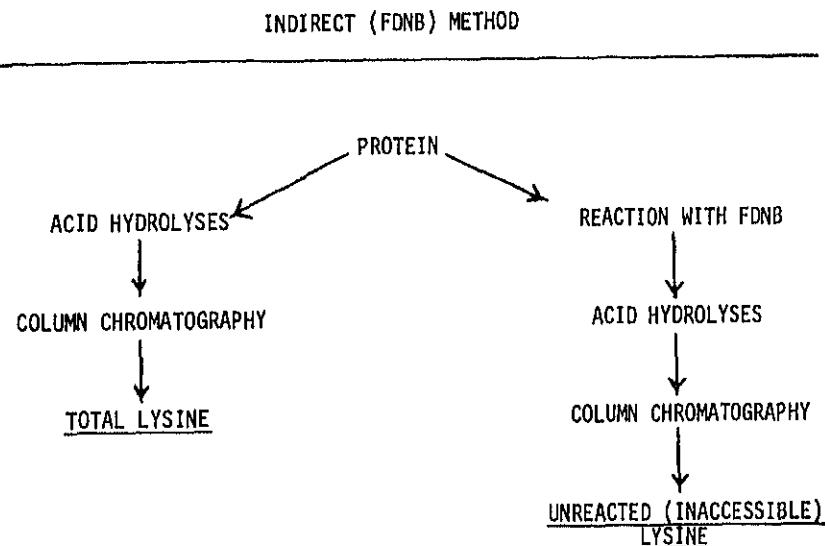


Figure 3. Schematic representation of procedures for estimating available lysine by the FDNB-indirect method.

For high carbohydrate foods, the direct methods (not considering the methylisourea and <sup>19</sup>F NMR methods) fail to yield satisfactory analytical

results. Use of the indirect methods appears to circumvent these analytical problems. However, the relationship of available lysine levels estimated by these methods and the nutritive value is not as satisfactory.

Relationship of Chemical Estimates to Nutritive Value. The large majority of the comparisons made between available lysine as estimated by chemical methods and available lysine as estimated by animal bioassays have been made in studies in which the direct FDNB procedure was used. Most of these comparative studies were made on meat or fish products used for protein supplementation.

In these studies, it is clear that a relationship exists between nutritive value and levels of available lysine as estimated by the FDNB direct method (1,2,9,10,13,40). For predictive purposes, however, the relationship is not as good as many reviews have implied. For instance, as shown in Figure 4, an N.P.U. (net protein utilization) value of about 30 was observed for several animal protein concentrates varying in available lysine between about 2-1/2 and 5 g/16 g N. In this same range of available lysine estimates, N.P.U. values as low as about 5 and as high as about 40 were also observed. Likewise, at a level of 6 g available lysine/16 g N, N.P.U. values of about 40 to 60 were observed.

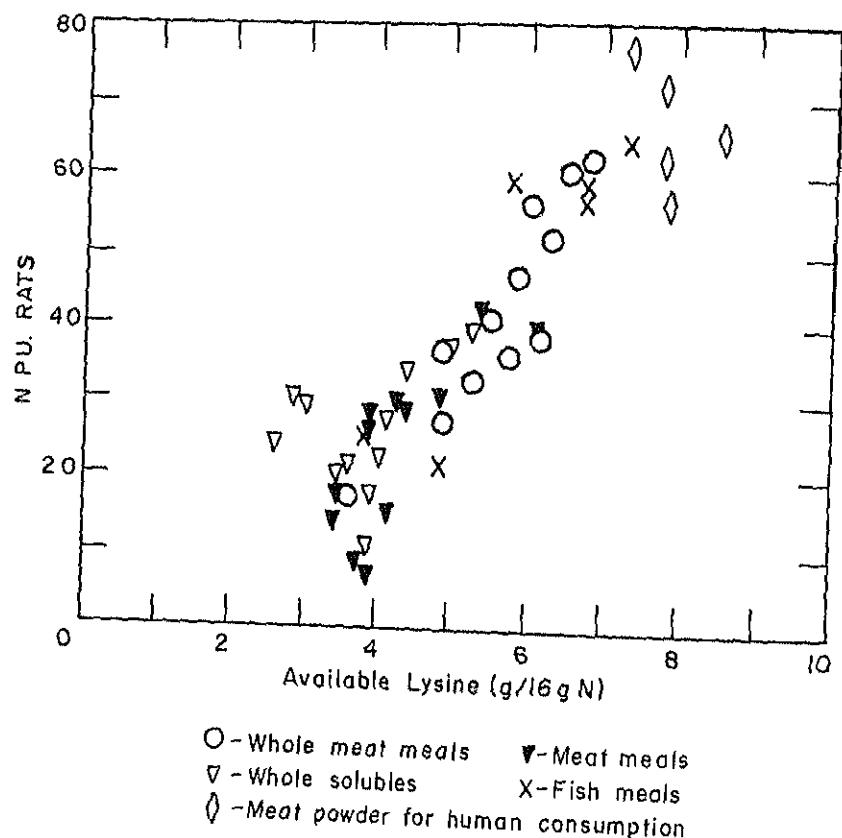


Figure 4. Relationship between N.P.U. (net protein utilization) values and FDNB direct method estimates of available lysine; data of Boyne et al. (2) and Carpenter and Miller (14). From NAS/NRC (39). Published with the permission of the National Academy of Sciences.

In a few studies, such as one conducted by Mottu and Mauron (36), a very clear-cut relationship has been observed in heat-treated protein sources such as milk (Table 3). With other proteins, including animal proteins, however, serious exceptions to such a relationship have been reported (e.g., see refs. 1,5,21). No studies have been reported which have attempted to relate availability of dietary lysine in human subjects with chemical estimates of available lysine.

TABLE 3. Percent Available Lysine in Four Milk Powder Samples As Estimated by FDNB (Direct) Method and By Rat Bioassay<sup>a</sup>

Total Lysine by Chemical Analyses (g/16g N)	% Available Lysine	
	FDNB Method	Biological Assay (Rat Growth)
8.0	103	101
7.6	84	80
6.8	56	59
6.1	31	33

<sup>a</sup>From Mottu and Mauron (36); four milk powders, with varying degrees of heat damage, ranging from "good quality" (top) to "severely scorched" (bottom).

Wheat and Wheat Products. Chemical estimates of available lysine in wheat and in wheat gluten, obtained by use of both direct and indirect methods, are listed in Table 4. The more reliable estimates, obtained by use of the indirect methods, are in general agreement. Thus, according to chemical estimates, the lysine in wheat is 85-100 percent available; in wheat gluten, 90-100 percent. Similar values are obtained for bulgur (Table 5).

TABLE 4. Chemical Estimates of % Available Lysine in Wheat or Wheat Gluten

	Wheat	Wheat Gluten	Reference
<u>Direct Methods</u>			
FDNB	79.5	90.0	Booth (7)
FDNB (corrected by % recovery of DNP-Gluten)	94.8		Booth (7)
FDNB (polarographic analyses of DNP-Lysine)	90.5		Blom et al. (4)
TNBS	43.0		Bodwell and Hagen (6)
<u>Indirect Methods</u>			
Total Lysine Minus Inaccessible (FDNB)	97.9 92.9	98.2	Booth (7) Bodwell and Hagen (6)
Total Lysine Minus Inaccessible (Methyl-acrylate)	86.3	95.2	Finley and Friedman (16)

TABLE 5. Chemical Estimates of Percent Available Lysine in Wheat, Bulgur, or Fortified Bulgur

	Wheat	Bulgur	Fortified Bulgur
<u>Direct Methods</u>			
FDNB	105.2	109.8	82.3
TBNS	43.0	43.4	32.2
<u>Indirect Methods</u>			
FDNB	92.9	85.2	97.9
Methyl Acrylate	86.3 <sup>a</sup>	86.0 <sup>a,b</sup>	-

<sup>a</sup>Data from Finley and Friedman (16); all other data from Bodwell and Hagan (6).

<sup>b</sup>Bulgur flour.

Estimates of the percent availability of lysine in wheat protein of wheat, flour, gluten, etc. (as determined by animal bioassay) are listed in Table 6. Since it is difficult to correct for endogenous amino acids and to allow for the effects of microbial action in the intestinal tract, the estimates obtained by fecal analyses should be disregarded. Most of the remaining values for estimated percent available lysine are in the range of about 70 to 80 percent. These bioassay estimates are markedly lower than the estimates obtained by using the chemical methods.

TABLE 6. Percent Available Lysine in Wheat Protein as Estimated by Animal Bioassays

	Wheat	Flour	Gluten	Germ	Bread
<u>Bioassay</u>					
Growing rats [carcass nitrogen] (8) <sup>a</sup>	75, 78	72, 80	80		76, 83
Growing rats [gain/food intake] (19)		70, 72			
Adult rats, depleted [gain/% dietary lysine] (20)				57, 72	
Rats [fecal analysis] (29)	93				
Rats [fecal analyses] (8)	74		99		

<sup>a</sup>References indicated by numbers in parentheses.

Since lysine is the limiting amino acid in most wheat products, it can be assumed that estimates of biological value obtained with experimental animals reflect (for the most part) the dietary level of available lysine. Although this assumption is not completely valid, it is useful to compare bioassay estimates of available lysine with estimates of biological value. For the rat (see ref. 3), whole wheat has a biological value of 60 to 70; wheat germ, 75, and wheat gluten, 40. These values are generally comparable to the previously discussed values of 57 to 83 for percent available lysine levels as estimated by rat bioassay.

For humans, in studies done 15-40 years ago, estimates of the biological value of wheat proteins were much higher than the values found for rats. Biological values of 80-90 for humans were reported for whole wheat (including toasted or shredded whole wheat cereals) and wheat germ (37,38) but a value of only 42 was observed for "wheat gum gluten" (22). Some of the more recent studies have resulted in values between 30 and 50 for both gluten and whole wheat proteins (26,46,51). Since the effects of caloric intake and the necessity of conducting experiments at near maintenance levels of protein intake were considered more carefully in the more recent studies, these estimates are more valid than those obtained in the earlier studies.

Conclusions. From the foregoing, it can be concluded that the estimates, obtained by using chemical methods, of 85-95 percent availability for lysine in wheat proteins are indeed probably overestimates for both rats and humans. The chemical estimates of available lysine may be overestimates because (a) in the direct methods, the previously discussed side-reactions would inflate the colorimetrically determined estimates of available lysine, and/or (b) in both direct and indirect methods, some of the nutritionally unavailable lysine may react with the chemical agents used to form acid stable derivatives and, if so, estimates of the available lysine level would be exaggerated.

For monitoring the effects of processing on a specific wheat protein product, chemical estimates of available lysine may provide a rapid approach for detecting large changes. For example, a percent available lysine value of 90 might be obtained with the unprocessed ingredients of a specific product and a value of 80 percent might be obtained routinely for the processed product. A value in a specific "batch" of product of 60 percent available lysine would then obviously indicate a malfunction in the processing procedures used for that batch. A decrease in lysine availability and/or some actual destruction of amino acids (including lysine) could be logically presumed to have occurred. Except in a general way, however, such values would not necessarily be an indication of nutritive value for humans.

In any case, lysine content or protein content may not be the most critical factors determining the protein nutritional value of wheat products for humans. As suggested by the unpublished work of Kies and Fox (28), one of the most critical factors affecting the nutritive value of wheat protein in human diets may be digestibility.

## Literature cited

1. Atkinson, J. and Carpenter, K. J. Nutritive value of meat meals II. Influence of raw materials and processing on protein quality. *J. Sci. Food. Agric.* 21: 366-373, 1970.
2. Boyne, A. W., Carpenter, K. J. and Woodham, A. A. Progress report on an assessment of laboratory procedures suggested as indicators of protein quality in feedingstuffs. *J. Sci. Food Agric.* 12: 832-848, 1961.
3. Bender, A. E. Chemical scores and availability of amino acids. In *Proteins in Human Nutrition*. Ed. by Porter, J. W. G. and Rolls, B.A., Academic Press, New York and London, pp. 167-178, 1973.
4. Blom, L., Hendricks, P. and Caris, J. Determination of available lysine in foods. *Anal. Biochem.* 21: 382-400, 1967.
5. Boctor, A. M. and Harper, A. E. Measurement of available lysine in heated and unheated foodstuffs by chemical and biological methods. *J. Nutr.* 94: 289-296, 1968.
6. Bodwell, C. E. and Hagan, S. N. Unpublished data, 1975.
7. Booth, V. H. Problems in the determination of FDNB-available lysine. *J. Sci. Food Agric.* 22: 658-665, 1971.
8. Calhoun, W. K., Hepburn, F. N., and Bradley, W. B. The availability of lysine in wheat, flour, bread and gluten. *J. Nutr.* 70: 337-347, 1960.
9. Carpenter, K. J. The estimation of the available lysine in animal protein foods. *Biochem. J.* 77: 604-610, 1960.
10. Carpenter, K. J. and Booth, V. H. Damage to lysine in food processing: its measurement and its significance. *Nutr. Rev.* 43: 424-451, 1973.
11. Carpenter, K. J. and Ellinger, A. M. The estimation of "available lysine" in protein concentrates. *Biochem. J.* 61: xi, 1955a.
12. Carpenter, K. J. and Ellinger, A. M. Protein quality and "available lysine" in animal products. *Poultry Sci.* 34: 1451-1452, 1955b.
13. Carpenter, K. J., Ellinger, A. M., Munro, M. I. and Rolfe, E. J. Fish products as protein supplements to cereals. *Brit. J. Nutr.* 11: 162-173, 1957.
14. Carpenter, K. J. and Miller, D. S. In *Evaluation of Protein Quality*, Report of an International Committee on Protein Malnutrition, Food and Nutrition Board, National Academy of Sciences-National Research Council Publication 1100, p. 7. National Academy of Sciences, Washington, D. C., 1963.

15. Concon, J. M. Chemical determination of critical amino acids in cereal grains and other foodstuffs. In *Protein Nutritional Quality of Foods and Feeds, Part I, Assay Methods-Biological, Biochemical, and Chemical*. Ed. by Friedman, M., Marcel Dekker, Inc., New York, pp. 311-380, 1975.
16. Finley, J. W. and Friedman, M. Chemical methods for available lysine. *Cereal Chem.* 50: 101-105, 1973.
17. Finot, P. A. and Mauron, J. Le blocage de la lysine par la reaction de Maillard. II. Proprietes chimiques des derives N-(desoxy-1-D-fructosyl-1) et N-(desoxy-1-D-lactulosyl-1) de la lysine. *Helv. chim. Acta* 55: 1153-1164, 1972.
18. Friedman, M. and Finley, J. W. Vinyl compounds as reagents for available lysine in proteins. In *Protein Nutritional Quality of Foods and Feeds, Part I, Assay Methods-Biological, Biochemical, and Chemical*. Ed. by Friedman, M., Marcel Dekker, Inc. New York, pp. 503-520, 1975.
19. Gupta, J. D., Dakroury, A. M., Harper, A. E. and Elvehjem, C. A. Biological availability of lysine. *J. Nutr.* 64: 259-270, 1958.
20. Guthneck, B. T., Bennett, A., and Schweigert, B. S. Utilization of amino acids from foods by the rat. II. Lysine. *J. Nutr.* 49: 289-294, 1953.
21. Hackler, L. R., Stillings, B. R., and Polimeni, Jr., R. I. Correlation of amino acid indexes with nutritional quality of several soybean fractions. *Cereal Chem.* 44: 638-644, 1967.
22. Hawley, E. E., Murlin, J. R., Nasset, E. S. and Szymanski, T. A. Biological value of six partially purified proteins. *J. Nutr.* 36: 153-169, 1948.
23. Holsinger, V. H. and Posati, L. P. Chemical estimation of available Lysine in dehydrated dairy products. In *Protein Nutritional Quality of Foods and Feeds, Part I, Assay Methods-Biological, Biochemical, and Chemical*. Ed. by Friedman, M., Marcel Dekker, Inc., New York, pp. 479-502, 1975.
24. Holsinger, V. H., Posati, L. P. and Pallansch, M. J. Anomalous results obtained in the determination of available lysine in casein using 2,4,6-trinitrobenzenesulfonic acid. *J. Dairy Sci.* 53: 1638-1639, 1970.
25. Hussein, L. A. Comparison of methods for the determination of available lysine value in animal protein concentrates. *J. Sci. Food Agric.* 25: 117-120, 1974.
26. Inoue, G., Fujita, Y., Kishi, K. and Niiyama, Y. Nutritive values of egg protein and wheat gluten in young men. Proc. IXth Intern. Congr. Nutr., Mexico City, September, 1972 (Abstract).

27. Kakade, M. L. and Liener, I. E. Determination of available lysine in foods. *Anal. Biochem.* 27: 273-280, 1969.
28. C. Kies and H. M. Fox. Personal Communication. 1975.
29. Kuiken, K. A. and Lyman, C. M. Availability of amino acids in some foods. *J. Nutr.* 36: 359-368, 1948.
30. Lakin, A. L. Evaluation of protein dye-binding procedures. In *Proteins in Human Nutrition*. Ed. by Porter, J. W. G. and Rollis, B. A. Academic Press, London and New York, pp. 179-193, 1973.
31. Matheson, N. A. Available lysine. I. Determination of non-N-terminal lysine in protein. *J. Sci. Fd. Agric.* 19: 492-495, 1968.
32. Matheson, N. A. Available lysine. II. Determination of available lysine in feedingstuffs by dinitrophenylation. *J. Sci. Fd. Agric.* 19: 496-502, 1968.
33. Mauron, J. Influence of industrial and household handling on food protein quality. In *Protein and Amino Acid Functions*. Ed. by Bigwood, E. J., Pergamon Press, New York, pp. 417-473, 1972.
34. Mauron, J. and Bujard, E. Guanidination, an alternative approach to the determination of available lysine in foods. *Int. Congr. Nutr.* 6, Edinburgh, pp. 489-490, 1963.
35. Mossberg, R. Estimation of protein content and quality by dye-binding. In *Evaluation of Novel Protein Products*, Proc. Intern. Biological Programme and Wenner-Gren Center Symposium, Stockholm, September, 1968. Ed. by Bender, A. E., Kihlberg, R., Lofqvist, B. and Munck, L., Pergamon Press, New York, pp. 203-209, 1970.
36. Mottu, F. and Mauron, J. The differential determination of lysine in heated milk II. Comparison of the *in vitro* methods with the biological evaluation. *J. Sci. Food Agric.* 18: 57-62, 1967.
37. Murlin, J. R., Nasset, E. S. and Marsh, M. E. The egg-replacement value of the proteins of cereal breakfast foods, with a consideration of heat injury. *J. Nutr.* 16: 249-269, 1938.
38. Murlin, J. R., Marshall, M. E. and Kochakian, C. D. Digestibility and biological value of whole wheat breads as compared with white bread. *J. Nutr.* 22: 573-588, 1941.
39. NAS/NRC (National Academy of Sciences-National Research Council). *Evaluation of Protein Quality*. Report of an International Conference Committee on Protein Malnutrition, Food and Nutrition Board. Publ. No. 1100, National Academy of Sciences, Washington, D. C. 1963.

40. Ostrowski, H., Jones, A. S. and Cadenhead, A. Availability of Lysine in protein concentrates using Carpenter's method and a modified Silcock method. *J. Sci. Food Agric.* 21: 103-107, 1970.
41. Posati, L. P., Holsinger, V. H., DeVilbliss, E. D. and Pallansch, M. J. Factors affecting the determination of available lysine in whey with 2,4,6-trinitrobenzene sulfonic acid. *J. Dairy Sci.* 55: 1660-1665, 1972.
42. Ramirez, J. E., Cavanaugh, J. R., Schweizer, K. S. and Hoagland, P. D. The determination of free  $\epsilon$ -amino groups of lysine in proteins using  $^{19}\text{F}$  NMR spectroscopy. *Anal. Biochem.* 63: 130-134, 1975.
43. Rao, S. R., Carter, F. L. and Frampton, V. L. Determination of available lysine in oilseed meal proteins. *Anal. Chem.* 35: 1927-1930, 1963.
44. Roach, A. G. Sanderson, P. and Williams, D. R. Comparison of methods for the determination of available lysine value in animal and vegetable protein sources. *J. Sci. Food Agric.* 18: 274-278, 1967.
45. Sanger, F. 1945. The free amino groups of insulin. *Biochem. J.* 39: 507-515, 1945.
46. Scrimshaw, N. S., Taylor, Y. and Young, V. R. Lysine supplementation of wheat gluten at adequate and restricted energy intakes in young men. *Am. J. Clin. Nutr.* 26: 965-972, 1973.
47. Seki, T. Chromatographic separation of DNP-Amino acids. *J. Biochem. (Japan)* 47: 253-258, 1960.
48. Tentori, L., Vivaldi, G., Carta, S., Velani, S. and Mandara, I. Automatic separation by column chromatography of ether- and water-soluble 2,4-dinitrophenyl derivatives of amino acids. Technicon Symposium, Automation in Analytical Chemistry, New York, N. Y., pp. 659-664, 1965.
49. Walz, O. P. and Ford, J. E. The measurement of "available lysine" in protein foods. A comparison of chemical, biological and microbiological methods. *Z. Tierphysiol. Tierernahr Futtermittelkunde.* 30: 304-322, 1973.
50. Williams, D. R. The determination of available lysine. 5th Colloquium on Amino Acid Analyses, Domont, France. Technicon International Division, Monograph 2, pp. 42-53, 1967.
51. Young, V. R., Fajardo, L., Murray, E., Rand, W. M. and Scrimshaw, N. S. Protein requirements of man: Comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef proteins. *J. Nutr.* 105: 534-542, 1975.

## HYBRID WHEAT VIEWED BY COMMERCIAL SEED COMPANY

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### Introduction

Of all grain crops capable of being hybridized, probably wheat has required the longest research effort. Since wheat is a self-pollinated hexaploid, the genetic complexity in developing the necessary cytoplasmic and fertility restoration systems, is more complicated than encountered in a cross pollinated diploid such as corn. In addition, wheat is used mainly for food rather than feed, therefore, the many desirable milling and baking characteristics must be incorporated into wheat hybrids.

However, since wheat is the world's number one food grain, grown on more than 500 million acres with yields averaging about 25 bushels per acre, it presents a worthy challenge. Adding 20 per cent to this figure, which we are now obtaining in our hybrids, an additional 2.5 billion bushels of grain could be realized annually.

Following the discovery of effective biological systems of cytoplasmic male-sterility and pollen fertility restoration in wheat, the possibility of hybrid wheat becoming a commercial reality took on a new light. Hybrid wheat research programs were started by public and private agencies throughout the world.

After the first surge in hybrid wheat research, many public and private agencies perhaps became discouraged with early results and drastically curtailed their research efforts. Yet, several commercial companies in the United States and a few public institutions have remained optimistic and have expanded their hybrid wheat programs. This persistence is now paying off as high yielding hard red winter hybrids of desirable type are being released on a limited scale.

Many of the earlier problems in producing hybrid wheat have now been overcome, yet, I would be naive in saying that we have solved all our problems. Rapid progress has been made in the past three to four years and should be even more rapid in the next five years since many problems have been solved and germplasms, techniques and facilities greatly improved. Our scientific knowledge of hybridizing self-pollinated hexaploids has also advanced through our research experience and new theories are being expanded continually. We believe hybrids should become an important economic reality by 1980, grown on thousands of acres in the United States and other countries as well.

### Advantages of Hybrid Wheat Proven

Most researchers have reported heterosis in wheat hybrids of the magnitude found in corn and sorghum. In hybrid wheat tests heterotic yield levels have been reported from a minus figure to fantastic levels of 100% increases; however, in the past few years when hybrids could be evaluated on larger field scales, indications are that hybrids are outyielding their parents by 15 to 30%.

Out of all wheat research have come certain conclusions generally accepted by hybrid wheat researchers.

1. High yielding parents with great genetic diversity be chosen to produce the best hybrids.
2. Second generation crosses ( $F_2$ ) have considerably less yield than first generation ( $F_1$ ) and thus are not economically practical.

We have developed shorter, superior strawed hybrids that are adapted to higher nitrogen applications and irrigation without the lodging problem encountered by earlier hybrids and many of our current leading standard varieties. Thus, a farmer can gain not only the genetic potential of hybrids, due to heterosis, but perhaps an additional 10 to 20 per cent yield from fertilization. Further yield advance can be expected as the stronger strawed hybrids are developed and grown under irrigation.

One of the major advantages of wheat hybrids over pure-line varieties is in the time involved in changing hybrids in case a new race of a disease hits, a change of one of the inbred lines could add resistance immediately rather than the years involved in developing a resistant pure-line variety. It is for this reason we are in the process of cataloging our R-lines and male-steriles on the basis of their various characteristics, allowing us to change inbreds as the need arises.

### Male-Sterile and Restorer Line Research

At the present time most researchers are using *T. timopheevi* cytoplasm as their main source of cytoplasmic male-sterility. However, we are aware of the danger in using one cytoplasm and a number of alien cytoplasms are now being investigated to evaluate their potential as additional sources of male-sterility and perhaps, may offer more to wheat hybrids than *T. timopheevi*, i. e., more vigor and additional genes for restoration.

During the past two years, we have compared yields and milling and baking quality of hybrids made with normal cytoplasm versus the same hybrids produced with *timopheevi* cytoplasm in order to learn if there are any detrimental effects of *timopheevi* cytoplasm. The results of this study are inconclusive but it does appear that yield is reduced by approximately 5% in

certain hybrid combinations by the sterile cytoplasm and no reduction in other combinations. There appear to be no difference in milling and baking characteristics. This study is being continued and expanded and soon additional hybrids with other alien cytoplasms will be evaluated.

One of the main reasons for the delay in the commercialization of hybrid wheat has been due to the complex problems in finding reliable pollen restoring R-lines that restore adequately under adverse environmental conditions and possess desirable agronomic attributes when used in hybrid combinations.

We also find considerable variation in the ease of restoration among our cytoplasmic male-steriles. These results indicate we need to search for different R-genes and combine them into a more complete restorer that would overcome the environmental and genetic variability problems.

Our assumptions are that we combine genetically different R-genes that appear to give additive effects. Also, it is theorized that perhaps the male-sterile system might contain modifier or complimentary gene(s) or even an inhibitor gene that was affecting the action of our earlier restorers.

It now appears our best restorer systems contain at least three major genes plus modifiers. We believe there may be a complimentary gene in both our male-sterile and restorer systems when full restoration is obtained in certain hard-to-restore soft winter and spring male-steriles.

#### Early Hybrid Wheat Research

Since the initiation of our research and development program, one of our major objectives was to develop larger spikes with more kernels per spikelet and retain good tillering capacity of our wheat lines. Many of our wheat lines, some of these are now in our male-sterile and restorer inbreds, exhibit these improvements. We feel by accomplishing this objective our future hybrids will express a higher degree of heterosis.

Our first experimental hybrids tested in 1970-71 were very disappointing in their performance. In a replicated test grown in three states only one hybrid out of 39 equalled the yield of the highest yielding check variety and only 14 equalled or exceeded the average of the checks. The checks were also shorter in height and earlier in maturity.

Perhaps similar data were found by other researchers and this might indicate why they became discouraged with the potential value of wheat hybrids.

However, we realized we had been selecting R-lines for their restoration ability only with little or no emphasis on yield, maturity, straw-strength and other desirable agronomic traits. This procedure seemed necessary because of the difficulty in isolating adequate restorers.

Significant expression of heterosis in hybrid wheat grain yields has generally been difficult to find. In our opinion, the performance of earlier hybrids was disappointing due partly to; 1) the incomplete fertility restoration system; 2) the low yield level of inbred lines making up the hybrids and/or; 3) too few hybrid combinations evaluated to find maximum heterosis.

For these reasons emphasis in our early research work was devoted to searching for more efficient fertility restoration systems and the accumulation of a diverse germplasm pool from which to develop superior inbred lines.

Realizing our early R-lines were poor wheat specimens other than their restoring ability, we initiated an active greenhouse program of transferring R-genes to high yielding normal height and semidwarf lines. Based on seed quality, we selected 200 experimental hybrids and planted them, 1970-71, in six replicated single rows along with six check varieties.

This test indicated the type of heterosis that could be expected if high yielding, good type R-lines were used in the hybrid combination. The average of the 200 hybrids was 14.6% above the highest yielding check variety and the average of the hybrids was 31.0% above the average yield of the checks. The highest yielding hybrid outyielded the highest yielding check by 41.6%.

While these data were from one year and one location only, they gave us the encouragement to expand our hybrid program.

Since 1971, we have made several thousand crosses and test crosses annually with the objective of developing high yielding, short-statured, cytoplasmic male-steriles and restorer lines of diverse germplasm and selecting for disease, insect and lodging resistance, winter hardiness and good milling and baking qualities.

We have sifted through the world collection of approximately 20,000 wheat lines and varieties from all parts of the world plus selecting and obtaining germplasm from many different sources. The crossing, selection and testing of wheat lines from these sources make up the nucleus of our conventional (pure-line) program. It is imperative that a strong pure-line breeding program be maintained in order to provide continuous superior inbreds for our hybrid program.

#### Current Hybrid Wheat Developments

During the past three years we have tested several hundred experimental hybrids and culled them heavily based on their performance. Many of these new hybrids are proving to be superior in both straw-strength and yield. Our most advanced hybrid test in 1974 contained 24 hybrids and eight standard varieties and was grown at 13 locations and in three to four replications. These data show that the average yield of the hybrids was 15.7% above the average yield of the standard varieties. Some hybrids exhibited a higher per cent of hybrid vigor at certain locations indicating where they are best

adapted. For example, the highest yielding hybrid at York, Nebraska, yielded 23.4% above the highest yielding standard variety.

Table 1 compares the four HRW hybrids we released last fall with eight varieties, 1973-74 data. The average yield of these four hybrids (HR900, HR925, HR975 and HR976) was 19.1% above the average yield of the eight standard varieties. The average lodging per cent was 29.3% for the hybrids versus 59% for the standard varieties. All four hybrids have good milling and baking characteristics.

Table 2, 1974-75 data, compares the four 1974 released hybrids, three released this fall, plus seven new hybrids that are either being increased for release or under test for possible future release, with seven standard varieties.

The average yield of the hybrids was 18% above the average yield of the varieties with the highest yielding hybrid outyielding the top variety by 15.9%. Averages for the hybrids and varieties were similar in maturity, test weight and per cent flour protein. Varieties averaged about one inch shorter than the hybrids while the average lodging was 49.6% for the hybrids versus 87.3% for the varieties. The hybrids exhibit a definite advantage over varieties in leaf rust and soil borne mosaic resistance.

The two year data indicate that the superior straw-strength of the hybrids would allow farmers to add additional inputs of fertilizer for further yield advance.

Table 3 is a summary of a high fertility test comparing varieties, advanced experimental lines (W558, W603, and W443) and two experimental hybrids (X3004 and X2172). The fertilizer applied was 168-72-34 lbs. per acre, and the harvested plot size was 5 ft. x 25 ft. Harvesting was intentionally delayed for two weeks to observe lodging and shattering of the entries. Over 50% of the wheat grown in Kansas is the variety Scout, and Scout derivatives. Scout has an excellent yield record which accounts for its popularity and its weakness is mainly in weak straw as evidenced by the low yield. In fact, Scout was 90% lodged before heading. These data show the need for straw-strength under high fertilization and under high genetic yield levels of some of the newer experimental lines and hybrids.

There has been much speculation regarding seeding rates of hybrids versus varieties. Several researchers have reported that the seeding rates of adapted hybrids could be reduced by as much as 50% without reducing yield, due to the heterotic expression of hybrids in emergence, vigor and extra tillering. Other researchers indicate hybrids do not yield better at the lower seeding rates. If hybrids do excel under higher seeding rates, it probably is due to heterosis expressing itself under high seeding competition.

We have conducted a three year yield test to compare the yields of entries seeded at 30 lbs. per acre versus 60 lbs. per acre. During 1971-72 and 1972-73 there was not a significant yield difference in seeding rates but the 1973-74 results showed a significant advantage for the higher seeding

Table 1. Summary-- Performance of Four Commercial Released Hard Red Winter Wheat Hybrids and Eight Standard Varieties, 1973-74

<u>Entry</u>	<u>Date Headed</u>	<u>Ht. inches</u>	<u>Per cent Lodging*</u>	<u>Leaf Rust**</u>	<u>Test Wt. Lbs/Bu***</u>	<u>Yield Bu/Acre***</u>
1. HR900	5/9	43	58	MS	60.0	37.7
2. HR925	5/13	44	39	MR-MS	59.7	37.5
3. HR975	5/14	47	10	MR	58.8	39.3
4. HR976	5/15	46	10	MR	59.2	37.6
5. Centurk	5/11	42	63	MS	59.6	34.6
6. Scout 66	5/11	45	56	MS	60.2	34.1
7. Parker	5/9	43	53	S	60.5	32.5
8. Trmp. 64	5/5	45	83	S	59.6	32.4
9. Sturdy	5/5	38	10	MR	58.5	31.6
10. Agent	5/14	46	29	MS	58.6	30.9
11. Satanta	5/11	40	89	S	60.2	30.4
12. Tascosa	5/12	44	89	S	61.6	29.1
Average yield of hybrids						38.0
Average yield of varieties						31.9
Average yield increase of hybrids over varieties						19.1

\*Average of three locations - Hutchinson & Assaria, Kansas and Enid, Oklahoma.

\*\*MR - moderately resistant.

MS - moderately susceptible.

S - susceptible.

\*\*\*Average of 13 locations in three states - Kansas, Oklahoma and Nebraska.

Table 2. Summary - Performance of 14 HRW Wheat Hybrids and Seven Standard Varieties, twelve locations  
In Kansas, Oklahoma, Texas and Nebraska, 1974-75

<u>Entry</u>	<u>Date Headed</u>	<u>Ht. In.</u>	<u>% Lodging*</u>	<u>Leaf Rust**</u>	<u>Soil Borne Mosaic***</u>	<u>Test Wt. lbs/bu</u>	<u>Yield bu/acre</u>	<u>% Flour Protein</u>
1. HR900	5/15	44	65	MS	3.0	60.5	38.9	12.2
2. HR925	18	45	49	MR-MS	3.5	59.7	39.1	12.1
3. HR975	21	47	42	MR	4.5	59.5	40.7	12.4
4. HR976	21	48	44	MR	2.0	59.3	37.9	12.8
5. HR908	16	44	39	MR	1.5	58.8	39.5	12.5
6. HR915	18	45	54	MR-MS	2.0	60.1	38.3	13.3
7. HR915A	17	44	52	MS-S	1.5	60.5	38.5	12.6
8. HR900A	15	42	58	MR-MS	2.0	60.3	38.6	12.9
9. HR925A	19	46	51	MR	1.5	59.4	40.3	13.0
10. X2172A	16	43	29	MR	2.5	58.5	41.7	12.1
11. X2180	15	44	67	MR-MS	1.5	60.3	40.0	12.7
12. X2037	16	43	54	MR-MS	1.0	59.4	39.0	12.1
13. X2175	20	46	61	MS-S	1.0	60.5	38.6	11.8
14. X2219A	15	44	29	MR	1.0	59.0	38.4	12.2
HYBRID AVERAGE		17.3	44.7	49.6	--	59.7	39.3	12.5
15. Tascosa	18	43	94	S	1.0	60.7	32.6	12.6
16. Centurk	19	43	88	MS	3.5	58.9	34.1	12.8
17. Eagle	19	42	93	S	4.5	58.8	33.5	12.7
18. Parker	17	44	85	S	5.0	60.1	31.8	12.7
19. Agent	20	47	75	MS	4.0	57.8	31.0	12.9
20. Danne	13	41	89	S	4.0	59.9	34.0	11.0
21. Sage	20	45	87	MS	5.0	59.3	36.0	12.6
VARIETY AVERAGE	18.0	43.6	87.3	--	--	59.4	33.3	12.5

Average yield increase of hybrids over varieties

18.0

\* Average of four locations - Weatherford, Oklahoma, and Hutchinson, Culver and Mt. Hope, Kansas.

\*\* MR - moderately resistant.  
MS - moderately susceptible.  
S - susceptible.

\*\*\* Rating scale 1 to 5 - 1 resistant and 5 susceptible.

Table 3. Summary of High Fertility Test Comparing Varieties, Experimental Lines and Hybrids\*

Hutchinson, Kansas, 1973-74

	<u>Yield</u> <u>Bu/A</u>	<u>Lodging</u> <u>%</u>
Parker	32.2	70
Scout 66**	22.1	100
Satanta	23.9	80
W558	46.8	15
W603	43.8	18
W443	45.3	20
X3004	34.7	20
X2172	48.8	20

\* Fertilizer rate: 168-72-34 lbs. per acre.

\*\* 90% lodged before heading.

Plot size: 5 ft. x 25 ft.

Harvest date: July 10, 1974 (Date ripe - June 27; left until July 10 to observe lodging and shattering).

rate in every entry except one hybrid. The experimental hybrids yielded significantly higher than the varieties at both seeding rates.

Yields of the check varieties (Scout 66, Parker, Triumph 64 and Tam 101) were lowered an average of 18% when the seeding rate was reduced from 60 lbs. per acre to 30 lbs. per acre. However, yields of the 16 hybrids were reduced by only 11% when the seeding rate was reduced. This appears to be an indication of the increased tillering ability of the hybrids. Hybrids may not lower the optimum seeding rate because of their larger seed size, but it seems logical to assume that the increased tillering ability may reduce the loss when a poor stand is obtained.

The seeding rate study for the 1974-75 crop year, as shown in Table 4, was handled as a population study involving seven hybrids and seven varieties, with rates of 15, 30, 45, 60 and 75 lbs. per acre based on an average seed size. This study was harvested at two locations in Kansas and two seeding dates, September 19 and October 17, at the Hutchinson, Kansas, location. At Hutchinson, hybrids and varieties all yielded significantly higher at the September 19 planting date when planted at the 15 lbs. per acre rate but above the 15 lb. rate all entries yielded higher at the later planting date. The hybrids yielded 11.5% higher than the varieties at the early date and 16.5% higher at the later date. There was not a significant yield increase for the hybrids above the 45 lbs. per acre seeding rate while the yield of the varieties at the late seeding date was still going up at the highest seeding rate.

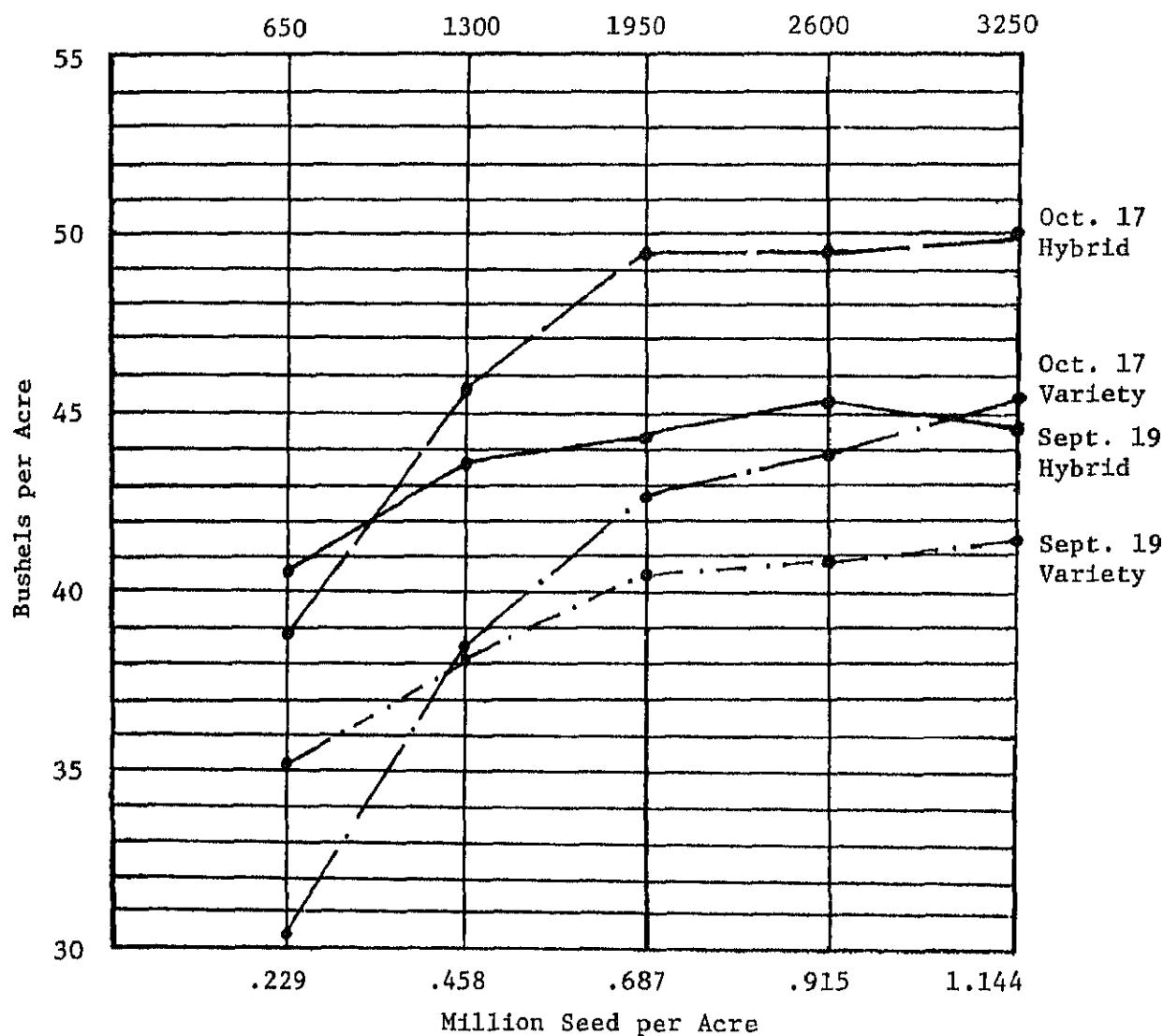
This same test was grown on dry land and planted September 23 at Garden City, Kansas. While the hybrids outyielded the varieties (average of all rate) by 14%, there was no significant yield advantage for either group above the 30 lbs. per acre rate.

A study was initiated in 1973-74 to determine if there is a yield advantage in wheat grown from large seed (usually found in hybrids) versus small or bulk (not sized) seed. Four varieties and two experimental hybrids were selected for the study and gram weights were taken of 1000 kernels of each size and each entry. The test consisted of eight replications with the plot size being 5 ft. x 15 ft. All plots were seeded at 800 seeds per plot and this is equal to 30 lbs. per acre assuming a weight of 35 gms. per 1000 kernels. Seed for the test was separated from the bulk by screening. There was a significant yield advantage in favor of the large seed over the small and bulk in every instance and a significant advantage of the bulk over the small size seed in all entries except the varieties, Danne and Centurk. Perhaps these results are magnified due to the low seeding rate.

Modified single crosses and three-way crosses are in yield trials to compare yields, at different locations, with true F<sub>1</sub> hybrids. If high yield levels can be maintained, there is a good possibility to add additional gene(s) for certain characteristics; in addition, there is a good possibility of increasing seed sets, thus, lowering seed cost.

Dual purpose winter hybrids are very important due to the need for pasture as well as grain. This is particularly true in areas where the

Table 4. 1974-75 Seed Population Study involving Seven Standard Varieties and Seven Experimental Hybrids



Plot size: 4.8' x 19.25'

Dates Planted: September 19 and October 17, 1974

Fertilizer: 200 lbs. of 16-32-8 per acre

Number of Replication: 4

environment promotes fall, early winter and early spring growth. We have never believed sterile forage hybrids would be profitable to the farmer, but we have continuously worked toward the development of dual purpose wheats.

Dual purpose hybrids and standard varieties were tested by clipping one and two times and harvesting the grain for yield. Several hybrids appear very promising for marketing as dual purpose hybrids.

We are also incorporating higher protein lines into our breeding program, such as the materials discovered and made available by the University of Nebraska plus lines obtained from breeders in other countries.

Due to the increase in damage caused by wheat streak mosaic and soil borne mosaic in recent years over a large area of the hard red winter wheat region, we are initiating a screening and testing program for these virulent diseases. Presently most of our new experimental hybrids have shown good resistance to the soil borne mosaic virus in all areas where they have been tested. Many of the leading varieties are susceptible or only have an intermediate tolerance to soil borne mosaic. Also, we are working with various fungal diseases including the rusts, smuts and mildew.

Plans are to expand our testing of hybrid combinations that tiller well under stress and still retain a good head size and seed number with the possibility of finding profitable hybrids for growing in the lower yield areas of the major wheat belts.

Since 1971 we have conducted numerous studies with chemical gametocides to evaluate their effectiveness in inducing male-sterility. Chemicals used have been Ethrel, UNI-D513, HR-531, HR-532 and HR-2956. Detrimental effects were noted with all chemicals tested, especially at the heavier application rates. Our results indicate these chemicals have little promise as wheat gametocides. In 1973-74, our studies with the chemical DPX 3778 have been more promising and additional tests will be implemented.

#### Factors Limiting Yield Advance

We have found in our search for the highest yielding inbreds for our hybrid program, that the highest yielding inbreds and hybrid combinations are derived from diverse germplasm. For example, we have a number of pure-lines, or inbreds, that have outyielded the leading hard wheat variety by over 30% four years in a row. Yet, we have not released these lines as varieties or hybrids made up of these lines due to their undesirable milling and/or baking characteristics. In addition to the much higher yield, these shorter, mostly semidwarfs, are far superior in straw-strength than standard varieties even though they are holding up the higher grain yield and also, have better disease resistance. As you are aware, some borderline or inferior varieties are now being sold on an increasing scale in certain areas. We have not gone in this direction. However, we are selecting some of the highest yielding inbreds and using them as either R-lines or male-steriles

and choosing their counterparts that are superior in milling and/or baking characteristics to see if they can complement each other and produce satisfactory quality characteristics. Such hybrids were grown in 1974-75 and are presently being evaluated by our cereal chemist. If they fail to meet quality standards, this means we have to make further crosses and selections to improve quality. We have found it is sometimes difficult to improve or maintain quality without sacrificing yield in the process, if additional crosses and especially backcrosses are necessitated.

To further complicate this problem, some hybrids and pure-lines are satisfactory one year and unsatisfactory the next, to illustrate the problem we face in our inability to control environmental influences.

Individual farmers are increasing their selection of seed wheat based on yield, desired maturity and standability and paying less attention to good milling and baking quality standards. Especially due to the price squeeze the farmer faces, means he will select the highest yielding wheats available to him. It is for this reason that I would stress that more research be implemented to reliable quick evaluation methods and reward the individual farmer who grows superior quality wheats.

The more characteristics we must breed for and the more complex the genetic makeup of the species we are working with, the more time and research expense is involved in making improvements.

There are indications that some research organizations are working on products that will differ from what we now call desirable milling and baking quality, whether dealing with bread or pastry wheats.

Are there milling and baking techniques available or could they be made available through research to permit some characteristic changes necessary to produce a quality flour, rather than depending so much on environmental and hereditary factors? This would be a break-through to allow plant breeders more leeway in breeding for yield and the many other hereditary traits he is presently dealing with in addition to the various quality characteristics.

For example, millers and bakers must be able to provide the breeder with more precise answers as to the type quality they will want ten years down the road. Varietal and hybrid development is too slow and complicated to change rapidly should desired quality characteristics change.

For these reasons I feel it is necessary for both groups, breeders and the milling and baking industry, to work close together to further research efforts in order to insure the continuation of producing high quality wheats combined with high yield and other phenotypically desirable traits.

#### Seed Set and Seed Production Techniques

The major seed production problem is in "nicking," that is the female being receptive when the male is shedding pollen. We realize it will be more

effort to maintain a uniform seed set in wheat than can be attained in corn and sorghum. Continued research on methods to improve nicking is necessary. Plant breeders are constantly searching for lines with large anthers that are well extruded before the pollen is shed and for male-steriles with perianths and lemmas that open wide and remain open for longer periods to be more receptive to the wind blown pollen. It is important that production fields are grown in areas where the wind velocity is high enough to carry the pollen to the females. Research has shown that the direction of planting is important in order to take advantage of the prevailing winds. Other necessary requirements for seed production are isolation from other wheats and land that was not previously in wheat, rye, or other contaminating crops where volunteer wheat is a problem.

Variations in seeding rates and seeding dates are used as methods of improving the "nick" between certain male-steriles and pollinators. In 1974 a test was planted to see if the "nick" in hybrid seed production could be improved by varying the following cultural practices:

- 1) Seeding rate; 20, 40, 60, 80 lbs./acre
- 2) Nitrogen level; 0, 25, 50, 75, 100 lbs./acre
- 3) P<sub>2</sub>O<sub>5</sub> level; 0, 20, 40, 60, 80 lbs./acre

Two male-steriles and two R-lines were included in the study at the above seeding rates. Fertilizer was applied at 25 possible combinations of N and P<sub>2</sub>O<sub>5</sub>.

Only minor trends were observed in the study in maturity and yield, indicating the study should be repeated with additional replications before definite conclusions can be made. It is questionable that fertilization can definitely be used in improving the "nick" between male-steriles and pollinators due to the many variable soil fertility levels and cultural practices encountered in the many areas of seed production. Also, environmental conditions vary so greatly from year to year that maturity predictions would be difficult to make.

High yield areas with mild temperatures and relatively high humidities, coupled with good cultural practices are more conducive to higher seed sets. For profitable seed production, we believe we need seed sets of 60% or higher with ratios of female to male at least 2:1 and preferably 3:1.

Other areas of seed production research involve the evaluation of blends in an effort to reduce seed cost and to retain as high as possible the degree of heterosis for yield. The blending of R-line (10-15%) would reduce the supervision necessary to the farmer who is producing hybrid seed for us and possibly increase the per cent seed set, thus, reducing the ultimate hybrid seed cost to the farmer and provide a more uniform and predictable seed quantity each year.

Various chemicals will be applied and evaluated as to their effect in speeding up or retarding anthesis to promote better nicking of A (MS lines) and B-lines and A-lines and R-lines.

## Summary

Hybrid wheat research is beginning to pay off as high yielding wheat hybrids of good agronomic type and with good milling and baking qualities are now being produced and marketed on a limited scale. Further improvement and more rapid expansion is expected in the next few years in both hard and soft hybrid wheat classes. Additional restorer (R-line) and cytoplasmic male-sterile systems are in the making. Many additional basic research areas are underway or being expanded to provide additional germplasm, improved techniques and knowledge for the development of better wheat inbreds and hybrids. Better cultural and management systems can be expected which will benefit both the hybrid seed wheat producer and the farmer growing the wheat hybrids. With these advancements we should see improvement in hybrid wheat progress similar to that which we have witnessed in corn and sorghum.

Varieties and hybrids, especially semidwarfs, will continue to be released at an accelerated rate. In my opinion, we can expect a more rapid advance in wheat yields in the decade ahead and the possibility that many of the higher yielding releases may be lacking in certain milling and baking characteristics should not be overlooked. Farmers will lean toward the higher yielding, better strawed wheats without regard to quality, unless there is a price incentive for him, as an individual farmer, to grow good milling and baking quality wheats.

In no way do I intend to leave the impression that commercial and perhaps public breeders as well, are not aware of the importance of good quality. I am merely pointing out some of the problems we face and hopefully, both groups, breeders and the quality industry, will further research areas that would be beneficial to both and at the same time provide higher yielding, more profitable wheats for our farmers.

There are still problems to be solved before we see wheat hybrids on vast acreages of wheat land. But with continued progress through research and development, and if the economic picture is favorable, the prospects for large scale commercialization of hybrid wheat are excellent. The monetary return the farmer can expect from hybrid seed wheat over varieties will be the ultimate determinant factor in hybrid wheat success.

THE FUTURE OF IRRIGATED WHEAT IN THE UNITED STATES

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Irrigated wheat production in the United States is almost exclusively in the Southern High Plains and in the Western States. In 1974, more than 1.5 million acres of irrigated wheat were harvested in the High Plains areas of Texas, Kansas, Oklahoma, Colorado, and New Mexico, and essentially the same number were harvested in the Western States; Idaho, California, Washington, Arizona, Utah, and Oregon, Table 1. The remaining 131,000 acres of a total 3,433,000 irrigated acres in the U.S. were harvested in Montana, Nebraska, Nevada, Wyoming, and North Dakota.

Table 1.--Irrigated wheat acreage in the United States, 1974<sup>1/</sup>

State	Harvested acres		Yield	
	Irrig. 1,000's	Non-irrig. 1,000's	Irrig. bu/A	Non-irrig. bu/A
Texas	1,000	2,300	21.1	13.8
Kansas	324	11,276	36.9	27.2
Oklahoma	129	6,270	24.7	20.9
Colorado	100	2,554	40.1	25.1
N. Mexico	90	71	27.4	5.0
	1,643	22,471		
Idaho	470	970	65.9	31.9
California	455	295	66.3	29.9
Washington	299	2,815	74.0	35.1
Arizona	235	—	66.0	—
Oregon	130	1,099	68.6	39.9
Utah	70	225	64.8	19.0
	1,659	5,404		
TOTAL	3,302	27,875		
TOTAL IN U.S.	3,433	62,026		

<sup>1/</sup> Texas Crop and Livestock Reporting Service.

Irrigated production in 1974 was 42.7 million bushels, or 7.4% of the total wheat produced in the five Plains States, Table 2. The 112.2 million bushels of irrigated wheat were 37.4% of all wheat produced in the six Western States. These two distinctly different areas accounted for nearly 96% of the total irrigated production and nearly 9% of all wheat produced in the United States in 1974.

Table 2.--Wheat production in the United States, 1974<sup>1/</sup>

State	Irrigated Million bushels	Total	Irrig/Total
			Percent
Texas	21.8	52.8	39.9
Kansas	12.0	319.0	3.7
Oklahoma	3.2	134.4	2.4
Colorado	4.2	67.8	5.9
New Mexico	2.5	2.8	87.0
	42.7	576.8	7.4
Idaho	30.9	61.9	50.0
California	30.2	39.0	77.4
Washington	22.1	122.0	18.1
Arizona	15.5	15.5	100.0
Oregon	8.9	52.8	16.9
Utah	4.6	8.8	51.7
	112.2	300.0	37.4
U.S.	160.9	1793.3	8.97

<sup>1/</sup> Texas Crop and Livestock Reporting Service.

It is obvious that wheat grown on the High Plains does not respond as well to irrigation water or that it is not irrigated as intensely as wheat in the western area. It is true that 1974 was a poor wheat year in the irrigated areas of Texas, New Mexico, and Oklahoma. However, a period of years' summary of average yields in Texas, Table 3, suggests that average irrigated yields for the Plains would not exceed 40 bushels per acre.

Table 3.--Wheat production in Texas, 1968-1975<sup>1/</sup>

	1000's of acres harvested		Yield, bu/A	
	Irrig.	Non-irrig.	Irrig.	Non-irrig.
1968	973	2155	37.0	16.3
1969	700	1751	37.0	19.4
1970	561	1706	37.0	19.7
1971	528	968	37.1	12.2
1972	447	1553	40.3	16.7
1973	865	2535	41.6	24.7
1974	1000	2300	21.1	13.8
1975	1330	4370	37.2	18.7

<sup>1/</sup> Texas Crop and Livestock Reporting Service.

Jensen and Musick (6) found seasonal evapotranspiration (consumptive use) for maximum wheat yields totaled 22-29 inches at Garden City, Kansas and Bushland, Texas. These data indicate that at moderate to high moisture levels winter wheat varieties in the late 1950's would produce about 120 lbs of grain per acre-inch of water. Musick et al. (11) found maximum efficiency was 2.4 bushels per acre-inch of water when seasonal water use was 18 to 22 inches at Garden City, Kansas. Grain sorghum grown with moderate to high moisture levels produced 2.5 to 3 times the amount of grain as wheat per acre-inch of water. A response similar to sorghum would have been made by corn.

Consumptive use data for wheat in Arizona (3), Idaho (8), and Washington (9), used with average irrigated yields, suggest water use efficiencies of 3 bushels or more (190 pounds) of grain per acre-inch of water -- a distinctly more favorable response than on the Plains.

The Southern Great Plains is underlain by the Ogallala formation, the primary source of irrigation water. There is very uneven distribution of this ground water resource in the 45 counties of the Texas High Plains, and with present use rates, the 340 million acre-feet of stored water in this area of the aquifer will be depleted by nearly two-thirds to 127 million acre-feet by the year 2020 (18). Depletion or lowering of water to uneconomical levels is the prospect much of the area must eventually face.

The first question is why did we have 2 million or more acres of irrigated wheat in the Southern Great Plains in 1975? -- Not what the future is for irrigated wheat in this area.

The wheat crop requires water primarily in the spring when needs by other crops like sorghum are minimal (6). In addition, irrigable land is in excess of that needed or which can be utilized for irrigated summer crops.

The Southern Great Plains is the only area where it is a common practice to use wheat both for grazing and grain production. In 1973, there were over 1 million head of feeder cattle on the area's wheat. Late August or early September sown winter wheat, which is adequately fertilized and watered, can produce as much as 1.5 tons of ovendry high protein forage by January 1. Shipley et al. (15) grew nearly 2.5 tons of ovendry forage by take-off time March 20 and more than 3 tons by April 30, Table 4. Forage produced with sprinkler irrigation at Texline was 11,000 pounds per acre during 9.5 months from August 15 to June 1.

Table 4.--Wheat forage yields and protein content, Texas Agricultural Experiment Station, North Plains Research Field, Etter, Texas, 1969-72.

Period of growth	3-year average	
	Ovendry forage lbs/A	Percent protein
Initial growth		
September 1 to December 3	1995	23.7
Regrowth		
December 3 to March 1	1915	25.3
March 1 to March 20	740	25.1
March 20 to April 10	695	24.1
April 10 to April 30	955	19.6
Total	6300	

Average daily gains of 400-pound steers placed on irrigated wheat pasture at Etter November 1 was 1.5 pounds per day from November 1 to March 20 and 1.8 pounds when grazing was continued through the higher production period to May 20, Table 5. At stocking rates of 1.5 head per acre, beef production was 293 and 515 pounds of beef per acre for the two periods.

Table 5.--Average daily gains of stocker cattle on irrigated wheat pasture, Texas Agricultural Experiment Station, North Plains Research Field, Etter, Texas, 1969-72.<sup>1/</sup>

Grazing period	Grazing days	ADG lbs	Lbs beef acre
November 10 - March 20	130	1.5	293
November 10 - May 20	191	1.8	515

<sup>1/</sup> Stocking rate/acre in November - 1.5 animals averaging 400 pounds each.

The high forage and beef production is not achieved without added cost. More water and fertilizer are required for wheat planted early for forage than for wheat sown later solely for grain production (15). In addition, early October seeded wheat has a greater yield potential than early September sown wheat, Table 6. With high beef and low wheat prices prevailing in the early 1970's, Shipley and Regier (15) concluded it was profitable to plant wheat early and graze until March 20 prior to harvesting a grain crop. During this same period, as much as 25% of the wheat in the Texas Panhandle was grazed until late May and grain was not harvested. Presently, with high grain and low beef prices, grazing may not be a profitable practice.

Table 6.—Effect of grazing on grain yields, Texas Agricultural Experiment Station, North Plains Research Field, Etter, Texas, 1970-72.

Grazing Period	Average yield of grain, bu/A
Non-grazed <sup>1/</sup>	62.0
Grazed to:	
March 1	44.0
March 20	49.3
March 30	45.0
April 10	45.8
April 20	33.0
April 17	21.8

<sup>1/</sup> October seeded wheat.

Double utilization of the wheat crop might be expected to insure profitable irrigated production. This was not necessarily true on the Texas High Plains in 1975 (12). Net return per acre was a minus \$31 at the average irrigated yield level of 37 bushels per acre and \$11 at the 50-bushel yield compared to net returns of \$18 and \$21 from above-average dryland yields in 1975, Table 7. However, income above variable costs was substantial and is used to pay fixed costs that occur whether irrigable land in excess of that needed or used for summer crops is irrigated or not. This is how irrigation of winter wheat is economically justified in the Southern Great Plains.

Table 7.--Estimated costs and returns per acre for wheat produced on the Northern High Plains of Texas, 1975<sup>1/</sup>.

	Furrow irrigated		Dryland	
	yield level, bu/A		yield level, bu/A	
	37	50	15	20
<u>Receipts</u>				
Wheat (\$3.25/bu)	\$120	\$163	\$49	\$65
Grazing (0.20/lb)	30	50	18	22
Total	150	213	67	87
<u>Costs</u>				
Variable	108	128	26	40
Fixed	73	74	23	26
Total	181	202	49	66
<u>Income above variable costs</u>				
	42	85	41	47
Net Returns	- 31	11	18	21

<sup>1/</sup> High Plains I 1975 Projected Budgets, Cecil A. Parker, Economist-Management, Texas Agricultural Extension Service, Texas A&M University.

The future of irrigated wheat production in the Southern Great Plains is dependent on reasonable income above variable costs. Should variable costs for natural gas, fertilizer, and other costs associated specifically with irrigated wheat increase faster than the price for wheat, irrigated wheat production will soon stop. However, if income after variable costs is reasonably high, irrigated wheat production will continue on the Southern Great Plains.

More efficient water use by wheat grown on the Plains may be possible. Schneider et al. (14) and Shipley and Regier (16) have shown that efficient use of water can be made by wheat grown with limited water. Timing of irrigation is very important with the most efficient use being made from irrigations during the fruiting period. Allen and Musick (1) found that growing wheat on 60-inch beds required less water but produced only slightly lower yields than that grown on 40-inch beds. Musick and Dusek (10) have shown that more efficient use of water can be made by growing sorghum and wheat in alternating double bed strips. Improved wheat varieties and experimental wheats, Table 8, may make more efficient

use of water than older varieties when grown under moderate to high moisture levels. Those that yield well under dry or wet conditions will be valuable where irrigation intensity cannot be predicted at planting or on dryland where there is great variation in annual rainfall.

Table 8.--Performance of commercial varieties and experimental wheats, USDA Southwestern Great Plains Research Center, Bushland, Texas, 1975.

Sel. No. or Variety	Yield		Plant height	
	Irrig. bu/a	Dry. bu/A	Irrig. inches	Dry. inches
Ks73112	99.6	31.0	29	20
Tx73A2798	94.2	35.8	28	21
Tx69A309-1	90.2	31.9	27	22
Tx69A569-1	90.1	33.8	28	22
Scout 66	81.5	32.0	33	24
TAM W-101	77.4	30.5	26	20
Sturdy	76.4	27.3	27	22
Centurk	71.7	32.8	31	21
LSD 5% Level	11.5	5.6		
C.V.	7.2%	11.5%		

New cultural practices, new varieties and even wheat hybrids may make it possible to extend the period of irrigated wheat production on the Plains. However, depletion of the Ogallala and higher energy-related production costs may preclude using irrigation water on Great Plains wheat in the not too distant future. In addition, where water resources become critical, government regulations and restrictions may place limits on use of water.

Irrigation water resources in the Western States are more plentiful, and availability of irrigation water can be projected in the more distant future than in the Southern Great Plains<sup>37</sup> (19). Wheat gives a greater response to irrigation water in the West than on the Plains. One-hundred bushel or higher yields per acre are common in most irrigated wheat areas of the West. This may be in part a result of growing relatively new, short, stiff-straw varieties suitable for high production levels. Dennis et al. (2) states that wheat now accounts for one-third of the irrigated acreage of Arizona and that acreage and yield increased dramatically since the introduction of stiff-strawed high yielding spring wheats developed by the International Center for Wheat and Corn Improvement and Winter Wheats from the Northwest. It is reported that 40% of the 1975 Arizona production was Durum.

<sup>37</sup> Comprehensive Framework Study, California Region, Appendix XVIII, General Program and Alternatives. Prepared by: California Region Framework Study Committee for Pacific Southwest Inter-Agency Committee, Water Resources Council.

A bright future is predicted for irrigated wheat in Arizona because of its high yield potential, suitability as a crop in rotations, and seasonal water requirements do not compete with water needs by other crops.

Improved varieties have played an important part in irrigated wheat in other western states. Qualset (13) credits stripe rust resistant "Mexican" wheats for increased yields in California. The value of white winter wheat varieties Gaines and Nu Gaines in the Pacific Northwest, their use in other areas, and value of other improved varieties is well documented.

Although irrigated wheat has relatively high yield potential, it also has a relatively low priority in many irrigated areas of the West. Horner (5) states that irrigated wheat is a residual crop of low priority in many parts of California and is being grown because it uses water when needs from other crops are minimal and because the lack of markets or contracts restricts the production of higher value crops.

Lindeborg (7) reported net returns from irrigated wheat yielding 80 bushels per acre in the Boise Valley of Idaho at \$62.19 and \$37.75 per acre for gravity and sprinkler systems, respectively, in 1975, if wheat sold for \$4.00 per bushel. However, if the selling price dropped to \$3.25 a bushel, net returns were only \$2.19 and a minus \$22.25 for each system. Nevertheless, fixed costs were \$100 per acre and substantial income was realized above variable costs. In 1974 in Fresno County California, production costs were \$5.26 per hundred weight or about \$3.16 a bushel for feed wheat yielding 67 bushels (4,000 lbs.) per acre<sup>4/</sup>. This yields a net return of only \$6.00 per acre if wheat sold for \$3.25 per bushel but substantial income over variable costs. Hathorn, in Arizona, (4) indicated \$5.38 per cwt (\$3.23 a bushel) was the break even price in 1975 for wheat yielding 4,300 pounds per acre with gravity irrigation, and with pump irrigation the break even price was even higher. Although net returns per acre were small at this yield level and price, income above variable costs was more than \$100 per acre for gravity irrigation.

Whittlesey and Butcher (19) indicate that, in general, any new irrigation development in the state of Washington will have to be supported by grain, seed and forage crops with relatively small amounts of fruits, potatoes and sugar beets. The latter crops, which have a much greater return per acre than wheat, face market restrictions that severely limit their production potential. Assuming high unit prices, Whittlesey and Butcher (19) projected net returns to water and management of \$855 for apples, \$1414 for sugar beets, \$1146 for potatoes, but only \$288 per acre for wheat. However, wheat would be grown on 30 to 50% of the land of suggested alternative rotations for irrigation development projects.

Irrigated wheat production may be more profitable in the West but, like on the Plains, wheat is grown with irrigation because it fits into rotations.

<sup>4/</sup> Irrigated Wheat - Single Cropped Cost Analysis Work Sheet 1974, California Agricultural Extension Service.

The overriding factor influencing future wheat production is the dependence of other countries on United States wheat exports for food.

Projections compiled in September by Smith et al. (17), of the Economic Research Service, USDA, include a need for 2,206,000,000 bushels U.S. wheat production in 1985, assuming a moderately high export demand. They also project that wheat yields will increase from the 1972-74 average of 32.6 to 37.9 bushels per acre in 1985. Using the projected 1985 wheat yield, it will take 58,205,804 acres of wheat to produce the projected 1985 needs. The indicated United States harvested wheat acreage in 1975 was 69 million acres and production from an average yield of 31.1 bushels per acre is 2,140,631,000 bushels, just slightly less than the projected needs for 1985. The product of 1975 indicated harvested acres and 1985 projected acre yields is 18.5 percent more than the 1985 projection. There appears to be little need to increase wheat acreage, even with more optimistic export projections. However, also included in these projections is an increase in fertilizer use. Nitrogen applications alone are projected to increase from 32 pounds per acre in 1974 to 47 pounds per acre in 1980. Should fertilizer not be available or be too expensive in 1980, projected yields would not be realized.

Irrigated wheat is not the most profitable crop. However, it is a valuable crop in that it fits into many rotations where other crops cannot be used, and its seasonal use of water does not compete with water needed for more responsive and higher value crops. New wheat varieties or hybrids may increase future yield potential dramatically in areas where irrigation water is plentiful. Increases of irrigated wheat production, if any, will be primarily in the West.

In conclusion, the future of irrigated wheat is dependent upon:

1. Availability of irrigation water as related to needs by other crops.
2. Net return per acre compared to those from other crops for which there is a market.
3. Relative efficiency of irrigated and dryland production.  
Increase in energy-related costs associated with irrigated production may make dryland production more competitive.
4. Dependence of other countries on wheat from the United States as a high protein food.

### Literature Cited

1. Allen R. R., and Musick, J. T. Wheat and Grain Sorghum Irrigation in a Wide Bed-Furrow System. *Transactions of the ASAE* 15(1):61-63 (1972).
2. Dennis, R. E., Thompson, R. K., Day, A. D., and Jackson, E. B. Growing Wheat in Arizona. *Arizona Agricultural Extension Service Bulletin* 169 (in press) (1975).
3. Erie, L. J., French, Orrin F., and Harris, Karl. Consumptive Use of Water By Crops in Arizona. *Arizona Agric. Exp. Sta. Tech. Bulletin* 169 (1965).
4. Hathorn, Scott. Personal Correspondence (1975).
5. Horner, G. L. Personal Correspondence (1975).
6. Jensen, Marvin E., and Musick, Jack T. The Effects of Irrigation Treatments on Evapotranspiration and Production of Sorghum and Wheat in the Southern High Plains. *Transactions 7th International Congress of Soil Science, Madison Wisconsin, U.S.A.* 1:386-393 (1960).
7. Lindeborg, Karl. Wheat Costs and Returns Per Acre, Boise Valley, Idaho (Mimeo budgets) (1975).
8. McMaster, G. M., and Larsen, D. C. Irrigation of Spring Wheat. *Idaho Agric. Exten. Ser. and Agric. Exp. Sta. Idaho Current Information Series No. 22* (1966).
9. Morrison, Kenneth J., and Fanning, Carl D. Supplemental Irrigated Wheat Production. *Cooperative Extension Service, College of Agriculture, Washington State University, EM 2962* (1968).
10. Musick, J. T. and Dusek, D. A. Irrigation of Grain Sorghum and Winter Wheat in Alternating Double-Bed Strips. *Jour. Soil and Water Conservation* 27(1):17-20 (1972).
11. Musick, J. T., Grimes, D. W., and Herron, G. M. Water Management, Consumptive Use, and Nitrogen Fertilization of Irrigated Winter Wheat in Western Kansas. *USDA, ARS Production Res. Rept. No. 75* (1963).
12. Parker, Cecil A. High Plains I 1975 Budgets. *Texas Agric. Extension Service (Mimeo report )* (1975).
13. Qualset, C. O. Personal Correspondence (1975).
14. Schneider, A. D., Musick, J. T., and Dusek, D. A. Efficient Wheat Irrigation With Limited Water. *Transactions of the ASAE* 12(1):23-26 (1969).

15. Shipley, John, and Regier, Cecil. Optimum Forage Production and the Economic Alternatives Associated with Grazing Irrigated Wheat. Texas High Plains. Texas Agric. Expt. Sta. MP-1068 (1972).
16. Shipley, John, and Regier, Cecil. Winter Wheat Yields with Limited Irrigation and Three Seeding Rates, Northern High Plains of Texas. Texas Agric. Expt. Sta. PR-3031 (1972).
17. Smith, Allen; Harrison, Virden; Yeh, Chung J.; Fox, Austin; and Quance, Leroy. Projections of the U.S. Farm Subsector and Policy Implications. National Economic Analysis Division, Economic Research Service USDA. Working Materials No. I.Q. 4.74 (1974).
18. Walker, Loyd, and Taylor, Howard. TWDB High Plains Study Shows 340 Million Acre-Feed to Water in 45-County Area. Water For Texas 5 (1 & 2):20-22 (1975).
19. Whittlesey, Norman K., and Butcher, Walter R. Irrigation Development Potential in Washington. College of Agriculture Research Center, Washington State University, Circular 579 (1975).

## WHEAT GLUTEN: THE CURRENT SITUATION

T. Frank Rawlinson, President - Centennial Mills,  
Division Univar Corporation

In my comments to you today, I carry two banners -- the first as president of Centennial Mills, a Division of Univar Corporation. The name "Univar" may be new to you so I will explain briefly. This is a Seattle-based corporation with such divisions as Centennial Mills, Penick & Ford, Van Waters & Rogers, VWR Scientific, Pacific Resins & Chemicals Company. Many of these names are, I am sure, familiar to you.

The Centennial Mills' Division is Portland-based, with mill properties in Spokane, Washington, Portland, Oregon and Los Angeles, California. We also own wheat starch/vital wheat gluten plants in Spokane and in Portland.

The other banner that I carry is that of a founding director and Vice President of the Wheat Gluten Industry Council. I will comment further on this Council of wheat starch/vital wheat gluten manufacturers later in my remarks.

On the chance that anyone here is not familiar with wheat starch or vital wheat gluten, I should like to describe each product briefly.

Through a process of washing a dough of wheat flour and water, it is possible to extract co-products. The starch is processed through a rather elaborate extraction system and then dried to a white powder for use in food, paper manufacturing, as laundry starch, fabric sizing, and many other applications.

Vital wheat gluten is extracted, further processed, and dried to a light-tan powdery product of 75% or 80% protein. The properties of vital wheat gluten are: its protein, its bland flavor, its ability to absorb water equal to 2 1/2 times its dry weight. Of great importance is its unique characteristic that we refer to as "vitality". This is the only high-protein cereal product that has this property of elasticity known as "vitality". Extreme care must be taken in drying the product to preserve this characteristic.

Vitality enables the gluten to provide strong cell structure in a baker's fermentation process. These strengthened cells capture the gases of fermentation to increase volume in the loaf and to permit addition of many ingredients -- such as raisins, rye flour, whole or cracked grains, and many others. This vitality also enhances the ability of this protein to adhere to breakfast cereals, to act as an adhesive to laminate meat products, and for use in the manufacture of artificial meat products. In all of these areas it adds the health-giving value of its high protein. The product -- vital wheat gluten -- is of further extensive value in the dietetic food industry.

As a prelude, at this point, to my comments on wheat gluten: "The Current Situation", and for background purposes, I would like to take a moment or two more to touch on the early days of this industry in Australia and the comparison of the general economics of the industry between Canada, Australia, and the United States. Canada and Australia are the two countries from which the major portion of gluten imported into the United States originates.

In 1923, following World War I, New Zealand imported wheat from Canada and Australia. It was necessary to blend high-protein Canadian wheat with the lower-protein Australian wheat to make a satisfactory loaf of bread. Bread was rationed in New Zealand due to supply of high-protein wheat.

Mr. Frank Hawker, of Christ Church, New Zealand, demonstrated the washing of a dough ball in a handkerchief under a faucet in his kitchen sink. He commented to a friend of the gluten remaining in the handkerchief: "This is the stuff that makes a good loaf of bread." Previously gluten had been manufactured in England; had been dried, but was devitalized in the drying process -- thus making it useless for improving bread quality since it had lost the elasticity that is so important in bread fermentation.

By 1933, Frank Hawker had developed a formula and the procedure to produce super bread using wet gluten. The gluten extraction, for addition to bread doughs, was performed by the baker under what was then called "The Proceria Process".

At about this time, the Proceria Company was formed with a world-wide patent covering this process. The procedure was for the baker to make a salt-free dough, allow it to rise, then develop it in a mixer, after adding salt and water. The fermented gluten rose in curds that were skimmed off, washed and mixed into the baker's dough. The starch and solubles were wasted and created a severe disposal problem.

In 1936, in Australia, J. B. Regan and Harry Flather successfully dried gluten without devitalizing the product. In 1939 Australian patent application 107603 was awarded to Regan and Flather for this drying process. In the meantime, during this 3-year period, several entrepreneurs had learned and copied the method. Mr. Regan has recently retired as Managing Director of the company that he founded in 1936 as a result of his development of the drying process -- Fielder's Starches of Australia.

Now take a look at the development of this industry.

In Australia wheat starch became a readily available product -- for paper coatings, fabric sizing, glucose production, and other industry and home consumption needs. The fact that little corn was raised in this country made wheat starch the base for nearly all starch needs. The Australian government imposed import tariffs to protect its wheat starch industry. The government further subsidized research and development of new products. They provided a subsidy to the wheat starch/vital wheat gluten industry to assure that wheat for raw material flour in this industry was competitive in the world market. They encouraged the development of world markets for gluten by offering travel incentives to the Australian gluten manufacturers. All this was designed so

that this industry could satisfy the starch needs of a growing country. In this protected atmosphere the starch/gluten industry grew to supply their starch needs and marketed their gluten around the world.

Canada, with a large supply of high-protein wheat and no corn crop, was also encouraged by the Canadian Wheat Board and other Canadian government agencies. As a result, Canada, who has little need for gluten in its baking industry due to its high-protein wheat crops, became a major exporter of gluten to England, the United States, and other world markets. The starch from this production found a readily-available market in Canada. Here again, there was an atmosphere encouraging this industry with a home consumption price on wheat and a willingness for government assistance of the expansion of Canadian wheat gluten plants. Only recently a starch/gluten plant in Canada was financed in part by a grant and a subsidy of some \$1,450,000 from agencies of the Canadian government.

Other countries, such as Germany, Switzerland, and Mexico, have been lesser factors in supply of gluten to the United States.

During the period since 1968 major factors in the world have provided a turmoil in the world gluten market. I will touch on these in the sequence of their happening.

The Kennedy Round of International Trade and Duty talks carried a theme of increased world trade. One of the results of this was a reduction of the duty levied on gluten imported into the United States. The duty was reduced from 20% ad valorem, by an amount of 2% each year over a five-year period, to its present 10% ad valorem basis. During this period gluten imports into the United States tripled. It is estimated that the increase of imports into the United States may be even larger than recorded by Customs districts. This assumption is based on knowledge that during 1973 and 1974, what I believe to be substantial quantities of gluten, containing 1% lampblack or 1% soya meal, or other ingredient, have been imported into the United States for use in the petfood industry. Gluten so imported was not classified as "gluten" by the exporter, but rather under another classification bearing a lower rate of duty or entirely free of duty.

It is estimated that during 1975 the import of gluten will reach 50% or more of total U.S. consumption. This compares to 1968 imports equal to 29% of U.S. consumption.

The second factor that has led to turmoil in the starch/gluten industry was the formation of the European Economic Community and the entry into this organization by England.

Perhaps due to the early origin of vital wheat gluten in England, the successful drying of gluten in Australia, the close working relationship between Australia and England in the import/export market, the need of England to import high protein wheat, and the large English market for high-protein breads, England has been a major customer for Australian and Canadian gluten export. The sharp increase in import duty levied by the European Economic Community has encouraged additional gluten production in England and in other

countries of the EEC. A common external tariff of 27% ad valorem, plus a variable levy, which changes daily, designed to protect the production of gluten in the EEC, is currently levied. As of July 10, 1975, this duty was \$13.40 per Cwt. Contrast this duty to the present U. S. ad valorem duty of 10%, or approximately \$3.70 per Cwt. It is small wonder that Canada and Australia have quickly recognized the possibility of reduced markets in the EEC and have therefore sought and enjoyed the rapid growth of the U. S. market and the opportunity to increase their exports to our market to the point that I have just mentioned, of obtaining an estimated 50% or more of the total U.S. market.

During this same period, the United States' gluten industry has been confronted with the additional problems of raw material cost and supply.

Our gluten industry has, for economic reasons, been based on the use of high-protein 2nd clears, from the milling industry, as its basic raw material. Only by use of this raw material can we hope to compete with the large and lower-priced corn crop and corn starch produced from this lower-cost corn.

For a period of time, as we had wheat surpluses in the United States, the gluten manufacturer could make use of the Processors' Certificate program. This program reduced his raw material cost on the portion of the end-product that was produced for non-human consumption. By pure coincidence, the end of the certificate program coincided with an increased demand for gluten in the United States. As a result, the U. S. gluten industry committed additional capital to growth and expansion. This brought about a heavier demand for 2nd clears at the same time as the Arab nations called on U.S. supply for an increased amount of export 2nd clear. This market pressure was increased further by the sharp increase in the price of wheat in the U.S. and the world markets brought about by heavy demand of wheat around the world. It is a matter of record that wheat, because of short supply, advanced in price at a more rapid rate than did corn.

The result to the U. S. wheat starch/vital wheat gluten industry was

Higher cost raw material,

Corn starch prices that lagged behind the rapidly increasing costs for wheat starch,

An increased importation of gluten products from subsidized raw material and by subsidized industries in Canada and Australia.

To put the frosting on the cake, so to speak, our industry at this point was forced to deal with increased demands from federal, state, and local Environmental Protection Agencies. These demands may challenge the very capability of this industry to comply.

There has been an increase of up to 300% in the cost of natural gas, which is the basic energy in the drying of both wheat starch and vital wheat gluten. In addition to this, the threat of service interruption in some areas

of the United States is very real. The sharp increase in electric power costs to operate these plants was a further blow.

It was in February of 1975 that as a result of the common problems facing our industry, the Wheat Gluten Industry Council was formed. This Council includes the 6 major wheat starch/vital wheat gluten companies of the United States and is open for membership to any commercial producer of gluten and wheat starch within the continental limits of the United States.

The purpose of the Wheat Gluten Industry Council is to present an industry voice to our governmental agencies in a legal and effective manner so that this industry can solve its problems and continue to exist. Among the actions taken by this Council in its first 8 months of existence are:

FIRST: An appeal to the U. S. Customs Classification and Value Division to prevent gluten from entering the United States' market at less than the 10% ad valorem duty. I referred earlier to the fact that in 1973 and 1974 gluten, with the addition of 1% lampblack or soya flour, had been entering this market under a classification other than gluten -- thus avoiding the 10% ad valorem duty. The petfood industry, to which this gluten found its way, represents the second largest outlet for gluten in the United States.

SECOND: An appeal to the International Trade Commission to prevent a further reduction in duty on imported gluten and a further pleading for a return to a level of 20% ad valorem so as to enable the U. S. gluten industry to exist. A new round of International Trade talks is beginning this fall. In the preparatory stages to this round of talks, the International Trade Commission, as well as special representatives from the office of Trade Negotiation, have permitted industry to present their case; arguments in support of, or in opposition to, reduction in tariffs on products imported into the United States.

THIRD: We are in the process of evaluating the Federal Environmental Protection Agency guidelines with a view toward considering industry action.

FOURTH: We are supporting the American Bakers' Association's request for the removal of current limitations placed on the use of vital wheat gluten in bread and rolls. Food and Drug Administration regulations currently limit the use of gluten to 2% in some cases, and 4% in others in the production of baked products.

Experience has now shown that there are many times when a higher level of vital wheat gluten is desirable in these products. The limitation should, therefore, be removed since vital wheat gluten is a healthful protein source made from wheat flour. Its addition in baked products should be free to rise to levels as needed by the baker, based on the product he wishes to produce and the economics of the use of gluten. Quality Bakers of America Cooperative has joined in recommending an increase in the permitted level of vital

wheat gluten in bread.

FIFTH: The Wheat Gluten Industry Council is exploring research potential for the development of new uses for vital wheat gluten. The value of this product is great. We feel that its potential is not fully known and understood.

In summary, please realize that many millions of dollars are invested by leading companies in wheat starch and vital wheat gluten plants. Our current problems are difficult. These problems must and will be solved.

To you, the wheat growers, the millers, the wheat agronomists, the scientists, the researchers, both from government and private industry, we represent a sizeable market. To provide our industries' raw material demands, we must have high quality, high-protein clears made from the best Dark Hard Winter and Dark Northern Spring wheats. An annual milling of some 126,000,000 bushels of the best wheats in these varieties is needed to provide the 2nd clear raw material used by the U. S. wheat gluten industry.

Your help is of great importance to us. Help to develop high quality good milling and good baking quality wheats from which our raw material, 2nd clear, is produced. Help in support of increased use of gluten to provide valuable protein in bread and rolls, in cereals, in petfoods, and as meat binders. Help in research of new uses or expanded uses of gluten in such areas as combination with soya protein to produce meat extenders and analogs. It has been learned recently that a combination of these two protein sources provides a protein efficiency ratio that exceeds that of either vital wheat gluten or soya protein as separate protein sources.

Other uses of vital wheat gluten that utilize not only its high protein content, but also its unique property of vitality, its bland flavor that enables its use to provide better bite in meat substitutes without detracting from basic product flavor.

We must also develop new uses and markets for wheat starch. Markets that permit the sale of starch at price levels that equate to our raw material cost rather than to other starches made from lower-cost raw materials.

I thank you for the opportunity to visit with you today. I encourage you to move forward with thought and vision to help develop new ideas of how we can expand the use of wheat through new and increased markets for vital wheat gluten. I would be pleased to have you contact me, by phone or letter, with ideas that may be conveyed to the Wheat Gluten Industry Council, to further develop this important utilization of wheat.

PROJECTS OF THE NATIONAL WHEAT INSTITUTE

James W. Coddington  
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Washington, D.C.

The staff of the Institute serves administratively only. All of its projects are conducted under contract by universities or private firms. The Board of Directors of the Institute decides what contracts to award and what proposals to reject. In the beginning, and as the work progressed, the Directors of the Institute have developed a concept or broad policy to serve as a guide to the direction of its efforts. While this concept or basis for its operations was not delineated in a treatise, as such, the gist of it threads through the records. This brief summary of the policy should help in an understanding of the thrust of the work.

A regular and adequate supply of the various kinds and qualities of wheat is of vital importance to the people of the United States and of the world. Food ranks high (near the top) among the material needs of mankind. It is my conviction, the matter of an adequate supply of food for all people, in all countries of the world has become, and will continue to be, more important in shaping world policy than gold, oil, or guns.

Wheat is a major source of food, both in developed and developing countries. World production of wheat, in 1975, has been estimated at about 370 million tons, or over 800 billion pounds. Truly wheat makes a significant contribution to the food needs of the world.

The United States has an adequate supply of the various kinds and qualities of wheat to meet the requirements of consumers in this country and to provide much more than the U.S. needs, actually billions of dollars worth, of wheat for export to other countries. If the United States is to maintain its role as an influence in progressing toward a better world for all mankind -- a world that cares for the needs of others, where hunger and malnutrition are minimized, and where peace reigns -- this nation must continue to be a major supplier of international trade in food for the world, especially a supplier of wheat of which we have a great capacity to produce.

No one should falsely conclude that we believe the United States can and should feed the world. On the contrary, we believe all countries should increase their production of food to help provide for a growing world population and to help drive hunger from the existing peoples of the earth. However, production forecasts of many other countries show continuing deficits of food, including wheat and other cereal grains.

The National Wheat Institute fully subscribes to the concept that the United States can and should continue to have a major role in developing world policy, including actions to minimize the problems of hunger and malnutrition, and of greater international trade, by expanding our marketings of wheat here and abroad. In fact this concept is expressed in the enabling legislation, the Wheat Research and Promotion Act of 1970. It was drafted and supported by wheat-producer organizations, and establishes "a program of

research and promotion designed to expand domestic and foreign markets and increase utilization for United States wheat .....". The National Wheat Institute was established to carry out the provisions of the above mentioned Act under an Agreement with the Secretary of Agriculture. The Institute is a non-profit corporation of producer organizations, and its only purpose is to administer the Wheat Research and Promotion Act.

Funding of the research and promotion programs is made from a total of slightly more than \$2 million left in an inverse subsidy fund and donated by wheat producers under provisions of the Act. The participating programs cover broad areas such as research in nutrition, marketing and transportation, market testing and development, and programs in wheat promotion and consumer education.

The first research contract, awarded by the Institute over three years ago, consisted of a survey of all known wheat research and promotion projects underway or completed in recent years. It identified areas needing further work and suggested some of the areas where expected results seemed most promising. Probably the greatest benefit of this "state-of-the-art" study was to identify many areas where a substantial amount of research had been previously carried out or was underway. The limited funds available to the Institute for its total program gave emphasis to the need to avoid a "shotgun" approach to awarding contracts and made it imperative that we "rifle in" on a few key issues which should have marked benefits now and in the future.

Effective wheat promotion and consumer education programs will have a bearing on the types of research efforts needed and their priority. Therefore, projects in such areas are mentioned as complimentary to our research actions.

For example, both the general public and the wheat industry should benefit by: (1) a more informed public of the importance of wheat to our national economy and the role that food products (including wheat) must play in shaping world policy to progress toward an era of peace; and (2) a more informed public of the specific attributes of wheat as food that causes it to be desirable in its own right. These conclusions have been instrumental in the Institute's decision to focus a substantial portion of its efforts (both promotional and research) on the specific desirable attributes of wheat products which, when fully recognized, should increase the demand for them.

The Institute has a number of consumer education and wheat promotion-type projects, which are highly successful. One of these projects, bringing outstanding results, is the Institute's financial support of the Agriculture Council of America. Reports of news media throughout the nation confirmed a great need for more factual information about the impact of wheat sales to foreign countries on the economy of this nation and the well being of individual consumers. The Agriculture Council of America is conducting a telephone "hotline" from the nation's capitol, in which toll-free phone calls are made from all over the U.S. with many different types of questions on this subject, and direct answers are provided by fully informed persons. This "hotline" and other means of communication has done much to dispell incorrect stories and opinions, and has helped to constructively show the plus values of U.S. wheat in international trade.

Two other promotion projects of the Institute focus on special desirable characteristics of wheat products. These are "National Sandwich Month and Contest" and "National Day of Bread" programs. These projects, as most Institute's projects, are funded in part from other sources. The two projects help to tell the story, with almost infinite variations, of how well bread may be used with other foods, to provide appetizing and nourishing meals. Television, radio, newspapers, consumer magazines and trade journals have repeatedly sent the essence of these programs into almost every home in America. Although a precise measure of the effectiveness of such blanket news media coverage, in terms of increased number of pounds of wheat products used, would be difficult to establish, there is little opportunity to doubt that many consumers have read and used the ideas put forth.

Facts about wheat and wheat products, to encourage increased marketings and usage, are reaching consumers and producers through means of a series of motion picture documentary films in color and sound. The first of these motion picture films produced by the Institute, entitled "Wheat Marketing - The Producer Has A Choice" has proved extremely popular and educational. Distribution has reached many thousands, based on requests from Maine to Florida, to California, and throughout the U.S., for showings to schools, colleges, clubs, organizations, foreign trade teams, groups and government agencies; and numerous requests for showings are continuing. The Council on International Non-theatrical Events has selected this film for its excellence to represent the United States in international motion picture events abroad and has awarded to it the Golden Eagle Award in 1974. The film will compete for an international award at the Ninth International Agricultural Film Festival, in Europe in early 1976. The film explores such market philosophies as direct selling, commission houses, farm bargaining, cooperatives, holding action, marketing orders, short and extended contracts, or a combination of these techniques. The role and use of futures and hedging actions is explained. The film makes no attempt to direct producers to any particular choice of marketing practices. Instead it shows clearly that the methods selected will not only affect the producer's price, but will materially affect the nationwide market for wheat.

Now in the midst of the shooting stage is a short (15 minute) film in sound and color being prepared for use in elementary schools, to develop an appreciation for good nutrition, and to call attention to the valuable nutrients in wheat foods. Some noted educators have expressed their conviction that good nutrition habits can be taught best when people are at elementary school ages. This film is intended to be used by school teachers to help good eating habits by the students. It is based on the premise that you can have more fun if you are healthy. It gives a balanced story on nutrition from various foods, but emphasizes the nutritional value of wheat foods. The script, reviewed and praised by government officials, is preliminary to a completed film, expected to be released about next April.

The Institute plans to reach many more American consumers in a third motion picture film which, as far as wheat products are concerned, faces up to the high cost of living problem uppermost in the minds of so many of us today. Its theme is that wheat foods are economical buys for consumers, in part, because of innovations which have been made in the distribution system to achieve improved efficiency of movement and handling. Records show that

although retail prices for wheat foods advanced in recent years, the rate of advance is less than that of a number of other commonly used foods. This film is in the midst of production and is expected to be released in March 1976.

A final motion picture film project of the Institute deals with international marketing of wheat. It shows the United States as a regular supplier of the various classes of wheat, of the qualities and quantities demanded by foreign buyers. The interdependence of the nations of the world is discussed. Food is shown to be of primary concern from a moral, political, and military standpoint. The great capacity of U.S. farmers to produce wheat makes it possible for us to export more than double, yes, almost treble, our domestic food consumption of this commodity. In fact, the film shows it is not only possible, but it is imperative that we export large volumes of wheat and other agricultural commodities in order to help give us the foreign exchange needed so we can import oil, TV sets, radios, cars and other products available from foreign countries. International wheat reserves is a part of the film, but mostly the film is promotional to help expand markets for wheat abroad. The film should be released in early 1976.

NWI wheat research projects are making commendable progress. Some of them have been discussed earlier on your agenda, including the work reported by Dr. Olaf Mickelsen, Michigan State University, and research at North Dakota State University as referred to by Dr. Kenneth A. Gilles, and I will mention such projects quite briefly.

The Institute is pleased to aid in funding research at Michigan State University designed to provide information on the nutritional and medical benefits from wheat in the human diet. Earlier research by MSU has indicated a relationship between wheat foods in the diet and blood urea levels. This study should provide a firm base for indicating the mechanism and possible implications of the reduced blood urea levels. The significance of the work is possibly threefold: 1. If the study provides scientific evidence that a diet in which most of the protein comes from wheat improves kidney function, then it may be possible to postpone the day when kidney patients must be subjected to renal dialysis. 2. The over-all effect of a diet high in wheat could have the effect of lengthening life by enhancing kidney function. 3. Evidence of previous research indicates the possibility that a diet containing a large amount of whole wheat products may be important in reducing the incidence of cancer of the colon. This research, now underway with human subjects, will endeavor to get a clearer understanding of why the wheat diet lowers the urea level of the blood and whether a wheat diet will help a person showing initial signs of renal disturbance to postpone the day he would have to depend on a kidney machine. The final results of this research are expected late in 1976.

A research contract, funded by the Institute, with North Dakota State University is now progressing to develop more nutritious pasta products from durum wheat. Work was begun two years ago on the 2-3/4 year study. Already two improved macaroni products have been produced in the laboratory. The new macaroni contains a minimum of 20 percent protein and meets existing

quality standards for color, firmness, cooking loss and cooked weight. These new macaroni products have been served in selected schools with a high acceptance rate. Consideration is now being given to additional market testing and commercial marketing of at least one of the new products. Other objectives of the contract include identifying those unique biochemical and functional properties which account for durum wheat's use as an acceptable pasta product, and developing potential applications for a wide range of foods and industrial products. In the latter area a number of potential snack food products have been developed.

The Institute is looking for more new wheat foods in a research contract with Colorado State University. The research is expected to pave the way for providing consumers with new food products from sprouted wheat. Such products would not only be rich in protein, but also in vitamins A and C. The vitamins mentioned are non-existent in the unsprouted whole grain or products therefrom. The study will investigate the ability of sprouted wheat to provide amino acids and other essential nutrients to laboratory animals. A search of the literature on previous studies indicates that the potential for utilizing sprouted wheat, especially for food products, has been grossly ignored. The recommendations for development of new food products are expected to be released about mid-1977.

A producer-owned firm, FAR-MAR-CO, has been awarded our largest research contract-- to develop an improved process to separate the components of the whole wheat kernel and to find satisfactory commercial markets for the various wheat components. The project goes beyond the laboratory stage. A large pilot plant has been constructed and the processing methods are now being tested for the manufacture of prime starch, vital gluten, bran, germ and germ oil. Reports show that very high quality prime starch and vital gluten have been produced in the pilot plant. Testing of procedures is underway for the processing of other products. An added value from this contract is an invention of new equipment for extracting starch, bran, and germ from wheat flakes. A patent application has been filed by the U.S. Department of Agriculture on this invention. The research study is progressing to find new uses for wheat. The most valuable part of the wheat kernel is the vital gluten and the wheat germ portion. Vital gluten is used to improve quality in bakery and cereal products. Adding gluten to bread-mix increases the final protein content, loaf volume and results in better crumb and texture. Gluten is also used in hydrolyzed vegetable proteins -- improving nutritional value and flavor of the resulting foods. It can be used as a whipping or foaming agent in foods (you may find it as the icing between layers of cake). It can be used as a meat extender or in many industrial products. Wheat starch can be further broken down into numerous products, including sugars, organic acids, antibiotics, ethyl alcohol, etc. This type of successful research means a whole new opportunity for wheat producers. They can specialize in producing different types of wheat for the various wheat products uses.

The pilot plant research has progressed so well that the Institute has entered an additional contract with FAR-MAR-CO to do a study of market potentials, and market testing of some of the new wheat products. The study is funded jointly by the Institute, Kansas Wheat Commission and FAR-MAR-CO.

It is designed to determine the commercial potential in terms of market opportunities for products developed from the components of wheat resulting from FAR-MAR-CO's new product development technology. The results of the evaluation of market potential are expected to be applied in a development and commercialization plan to place new wheat products in commercial marketing channels.

The several research contracts of the Institute, mentioned above, which are directed toward developing new wheat foods, feeds, and industrial products could well pave the way for a substantial increase in demand for wheat for use in processing such products for distribution in the United States and in foreign countries.

The Institute has a continuing concern for the role of wheat products in contributing to good health. It has a research contract with the American Institute of Baking to study the effect of wheat foods on cholesterol levels in blood. Studies indicate wheat and other cereals in the diet reduce cholesterol levels. Since heart disease is associated with high cholesterol levels, wheat based foods could play an important role in its prevention. The American Institute of Baking is trying to discover what component of wheat has this beneficial effect. If this component of wheat can be discovered and isolated, it could have a tremendous effect on the demand and use of wheat based products. It could also have a far-reaching impact on maintaining the normal levels of cholesterol in our blood, and consequently of good health and longer life.

Now that we have substantial research work progressing to develop new products from wheat, the Institute has contracted with the three state universities here in the Pacific Northwest (University of Idaho, Oregon State University and Washington State University) in a united regional project to discover new varieties of wheat which are more nutritious than existing commercial wheat varieties. In this research, hundreds of tests are being conducted on selected varieties from all classes of both winter and spring wheat. New "Promising" varieties produced in test plots by State agricultural experiment stations are compared with the leading varieties of each class of wheat now produced in the major wheat producing areas of the United States. It is primarily intended that results of this research will provide wheat breeders and agronomists with nutritional data to guide them in improving the nutrient content of wheat varieties, and also to provide guidelines for discouraging the release of new varieties of wheat with inferior nutritional value. The various classes and varieties of wheat are known to differ markedly in nutrient composition as well as functional properties related to milling and baking end-uses. Faster methods for determining differences in nutritional values are being developed and tested under this contract and will be made available to all who wish to use them. Evidence already exists, clearly indicating that some varieties of wheat are far superior to others in their ability to support animal growth. The basis for this observation is being investigated under this Pacific Northwest regional project. The work is assigned to each of the three universities in such a way that each university works on a portion of each sample of wheat selected for investigation. For example, Idaho is analyzing the samples for Vitamin E, total fat, amylase inhibitor and special-

ized animal experiments. Oregon analyzes samples for protein, amino acids, certain vitamins, and minerals. Washington is performing bioassays of the samples to determine growth response of each variety of wheat studied, and to determine whether certain growth-inhibiting substances are present in different wheats. Selected samples are being milled and made into baked or cooked products to demonstrate the carrythrough of these values. This research, which is now in its second year, holds great potential, because of the extensive use of wheat for food throughout the world. As the leading wheat varieties produced are improved in nutritional values, the nutrition of million of wheat consumers can be enhanced.

In conclusion, the program of the Institute potentially affects most people in the United States and many million of people abroad. I am convinced that its benefits are already many times the relatively small investment made. Furthermore, its benefits should increase in the future as the results of the research projects, and the education and promotion efforts are more widely applied.

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